New Zealand Journal of Agricultural Research

VOLUME 3 No. 4

AUGUST 1960

COMMONWEALTH AGRICULTURAL BUREAUX
16 MAR 1961

NOTICE TO CONTRIBUTORS

General

Papers published, or offered for publication, elsewhere are not acceptable. Nevertheless, publication elsewhere of an abstract or extended summary does not preclude publication in full in this journal.

Typescript

The original and one carbon copy are required, on one side only of foolscap paper, double spaced, with a left-hand margin of at least one inch and a quarter. Reasonably heavy good-quality paper should be used; flimsy paper delays the machine operator and consequently increases the cost of printing.

A brief summary is required, at the beginning of the paper. It should indicate the scope of the paper and give the principal results, and should be suitable for reproduction by abstracting journals as it stands.

All matter to be printed in italic type (e.g. generic and specific names) must be underlined.

Tables

Tabular matter costs more to print than letterpress and should therefore be kept to a minimum, bearing in mind, however, that a well planned table can take the place of a much greater area of letterpress.

Wherever possible, tables should be arranged to fit upright on the page, not sideways. They will be printed in 8-point type, and must be limited in size to fit the printed page, i.e. $4\frac{1}{4} \times 7$ in. maximum.

All tables, however brief, should be numbered, in arabic numerals. Numerous very small tables should be avoided.

Titles should be as brief as possible.

Units of measurement should be placed in parenthesis at the head of the column, not in the body of the table. Descriptive notes should be kept to the minimum, and abbreviations used wherever possible. For abbreviations, etc., the usage followed is that of British Standard 1991, Letter Symbols, Signs, and Abbreviations. Part 1. General.

Figures

These are to be numbered consecutively in arabic numerals, regardless of whether they are half-tones (photographs) or line blocks (graphs, etc.). Each must be referred to in the text, and only such figures as are essential to elucidate the text can be published. Graphs showing the same data as tables are not accepted.

The author's name, the title of the paper (abbreviated), and the figure number, should be written lightly in soft pencil on the back of each figure.

The maximum size for any figure is $4\frac{1}{4} \times 7$ in., less the space required for the caption.

Photographic prints should be on glossy paper, and may be "same size" or larger than the printed picture. Components of a composite figure should be firmly mounted on white card, and lettered as required A, B, C, etc.

Graphs should preferably be drawn about twice the final size, with the curves evenly inked throughout. Great care should be taken to ensure that all detail, especially lettering, is of such size as to be clearly legible when reduced to the final size. If he wishes, the author may insert lettering in pencil for re-draughting in this office.

If the scale is important, it should be shown on the figure, or, alternatively, the magnification or reduction may be stated in the caption, taking into account the printing reduction.

The figure number should not appear on the figure itself, only in the caption. Captions for all figures should be typed together at the end of the paper.

References

In the text, citations are to be by author's name and year, e.g. "(Brown 1957" or ". . . . as stated by Brown (1957)". The list of references at the end of the paper is to be arranged in alphabetical order of authors' surnames. Titles of papers cited may be included or omitted; if included, all words other than conjunctions and pronouns are to have capital initial letters. Titles of periodicals are to be abbreviated in accordance with the "World List of Scientific Periodicals" 3rd ed. 1952. The title of the periodical and the volume (and part) number are to be underlined for italics, but not the page number(s). Titles of books are to be in inverted commas and not underlined; the name and location of the publisher are to be given. For arrangement, punctuation, etc., of references, see examples in this issue.

NEW ZEALAND JOURNAL
of
AGRICULTURAL RESEARCH

Department of Scientific and Industrial Research, Wellington
Editor: M. O'Connor.

VOL. 3 AUGUST 1960 Number 4

PRELIMINARY ASSESSMENT OF TOXAPHENE, STROBANE AND THIODAN FOR CONTROL OF CLOVER CASE-BEARERS (*Coleophora* spp.) (COLEOPHORIDAE, LEPIDOPTERA)

By J. M. HOY, Entomology Division, Department of Scientific and Industrial Research, Palmerston North

(*Received for publication, 29 April 1960*)

Summary

In an unreplicated field experiment using areas 5 acres and larger for each treatment, 15 lb of toxaphene per acre in 3 applications, applied to white clover in flower, increased seed yields approximately 5 times and reduced seed damage caused by *Coleophora* spp. from 29.7% to 12.1%.

In laboratory tests, toxaphene and strobane at 10 lb/ac equivalent did not cause significant mortality in final instar larvae of *Coleophora alcyonipennella?* Kollar after 48 hours exposure. Under the same conditions, thiodan at 5 lb/ac equivalent caused 60% larval mortality.

Toxaphene and strobane are apparently highly toxic only to adults of *Coleophora* species; these materials may be used while clover is in flower with minimum risk of toxicity to honey bees. Thiodan may be toxic to honey bees when used on flowering crops.

Further trials using toxaphene and strobane appear warranted.

INTRODUCTION

Clover case-bearers, *Coleophora spissicornis* Haw. and *C. alcyonipennella?* Kollar have become major limiting factors to the production of white clover seed in New Zealand. Kelsey (1958) reported that in areas inspected in Ashburton County, white clover seed damaged by *Coleophora* spp. represented nearly 63% of all seed set. Palmer-Jones *et al* (1958) estimated the loss caused to seed growers by clover case-bearer at between £60,000 and £105,000 annually. The incidence of clover case-bearer damage has been the principal reason for decline in acreage of white clover seed harvested in Hawke's Bay. In recent years these insects have been responsible for serious damage to white clover seed in Mid- and South Canterbury.

Earlier investigations of chemical control of these *Coleophora* spp., as yet unpublished, indicated that insecticides such as DDT and lindane, were unlikely to give control of overwintering larval populations in the soil. Attention then turned to the possibility of applying insecticides to the white clover crop at flowering time while adults of *Coleophora* spp. were laying eggs. This paper gives an account of preliminary experiments using insecticides on the flowering crop.

Before this work could be undertaken, however, it was necessary to screen and field test suitable insecticides for their effect on honey bees working the white clover flower. This investigation was undertaken by Mr T. Palmer-Jones of the Department of Agriculture and has been reported elsewhere (Palmer-Jones 1958 and Palmer-Jones *et al* 1958). The preliminary field assessment of toxaphene and strobane against *Coleophora* spp. was conducted at the same time as the field investigation of the toxicity of these insecticides to honey bees on white clover flowers.

The effect of toxaphene, strobane and thiodan on final instar larvae of *C. alcyonipennella?* was assessed in a series of laboratory tests.

Field Experiments

The field experiments were carried out during the period December 1957 to March 1958 on white clover pastures on the property of Mr D. W. Van Asch at Te Awanga, Hawke's Bay. The trials were designed to meet the requirements of the Department of Agriculture in testing toxaphene and strobane for toxicity to honey bees. The assessment of these insecticides against *Coleophora* spp. was incidental to the above work. For this reason the trials were not replicated. A full description of plot layout and methods of application of insecticides has been given by Palmer-Jones *et al* (1958).

Toxaphene treatments

The pasture on which these trials were laid down was sampled for overwintering *Coleophora* larvae during September 1957. One hundred 2-in. cores at random gave a population level below 0.1 larvae per square foot. Sweep sampling of pasture foliage on 5 December yielded a mean of 2.8 adult *Coleophora* spp. per 20 sweeps.

Toxaphene 50% w/v emulsion was applied from the air to 12 acres on 8 December at the rate of 5 lb actual toxaphene in 10 gal of water per acre. Because of weather conditions there was no bee activity during the remainder of that day nor on 9 December. A further application of toxaphene at the rate of 5 lb per acre was sprayed over the 12 acres early on the morning of 10 December. To assess the effect of toxaphene dust on honey bees a 10% toxaphene dust was applied by hand to 7 of the 12 acres on 20 December at the rate of 5 lb actual toxaphene per acre.

Sweep samples were taken on all areas during the day and night of 10 December, day samples on 12 December and a final day sample on 7 January 1958. The results of these sweeps are shown in Table 1.

TABLE 1. FOLIAGE SWEEPS FOR *Coleophora* spp. ON TOXAPHENE TREATED AREA

Date	No. of *Coleophora* per 20 sweeps		
	No treatment	Toxaphene 10 lb/ac*	Toxaphene 15 lb/ac†
5.12.57 Day	Prior to treatment	— 2.8 adults	
10.12.57 Day	13.0 adults	1.6 adults	—
Night	11.9 adults	0.6 adults	—
12.12.57 Day	10.6 adults	1.0 adults	—
7.1.58 Day	45.4 adults + 4.4 larvae	12.5 adults + 0.1 larvae	5.9 adults

* 5 lb toxaphene on 8.12.57 and 5 lb on 10.12.57.

† As above plus 5 lb toxaphene on 20.12.57.

On 11 March 1958 the area was again visited and eight random collections, each of 100 heads of white clover, were taken on each of all treatments. In addition to using as a control strip an area on the hive side of the field parallel to the direction of aerial spraying, a further area at the tree end of the field (cf. Palmer-Jones *et al* 1958) which had received a limited amount of toxaphene during spraying was also sampled. When the clover heads were dry, the seed was rubbed out, cleaned and weighed. A random sample from each collection was examined microscopically to determine the percentage of seed damaged by *Coleophora* larvae. The mean seed yields per 100 heads and the percentage damaged seed are shown in Table 2.

TABLE 2. YIELD OF WHITE CLOVER SEED PER 100 HEADS AND PERCENTAGE DAMAGED SEED DUE TO *Coleophora* spp.
(Mean of 8 random collections of 100 heads per treatment)

Treatment	Weight in grams	S.E.	% damaged seed
Toxaphene 15 lb/ac	5.45	± 0.16	12.1
Toxaphene 10 lb/ac	3.85	± 0.16	13.2
No treatment (Tree end)*	2.66	± 0.16	—
No treatment (Hive side)	1.06	± 0.16	29.7

F ratio—132.7** for (3, 28) d.F.

* This area received a limited amount of toxaphene during aerial spraying on 8 and 10.12.57.

Strobane treatment

Strobane 40% w/v emulsion was applied to 8 acres on 16 December 1957 at the rate of 5 lb actual strobane per acre in 10 gal of water. Sweep samples were taken on 6 January 1958 when 2.4 adults and 1.8 larvae per 20 sweeps were present on the treated area and 3.7 adults and 8.1 larvae per 20 sweeps on the untreated portion of the paddock. This area was harvested for white clover seed at the end of February so no seed yield figures can be presented for the strobane treatments.

LABORATORY TESTS

Uninfested white clover flower heads on potted plants were sprayed on 29 January 1958 with toxaphene at the per acre equivalent rate of 2 lb of toxicant. A further series of heads was sprayed at the same dosage rate on 20 February. After this spraying, 5 sets of 5 flower heads were selected at random from each treatment and placed in petri dishes, each with 5 final instar larvae of *Coleophora* spp. Untreated flower heads were infested and used as controls. The mean survival rates after 90 hours exposure were as follows:—

Untreated heads	5.0 larvae
Heads sprayed 22 days previously	5.0 larvae
Freshly sprayed heads	4.6 larvae

The effect of toxaphene on uncased larvae of *Coleophora* spp. was assessed by removing final instar larvae from their cases and placing the larvae in petri dishes on filter papers sprayed with toxaphene at equivalent rate of 2 lb actual toxicant per acre. Five uncased larvae were confined in each of 5 dishes and a similar number on untreated filter papers. After exposure for 48 hours the mean survival rates were: untreated—5.0; treated—4.0.

Eight hours exposure of adults of *Coleophora* spp. to 2 lb acre equivalent of toxaphene resulted in 100% mortality compared to 28% in control. Strobane and thiodan were not available for laboratory testing against adults.

TABLE 3. DIRECT EXPOSURE OF CASED FINAL INSTAR LARVAE OF *Coleophora alcyonipennella?* TO INSECTICIDES ON FILTER PAPERS, 5 REPLICATES EACH WITH 5 LARVAE

Dosage Rate (per acre equiv., actual toxicant)	Mean rate of survival after 48 hours		
	Toxaphene	Strobane	Thiodan
No treatment	4.8*	5.0	5.0
½ lb	not used	not used	4.2
2 lb	5.0	4.8	3.8
4 lb	4.2	not used	not used
5 lb	not used	4.6	2.0
10 lb	4.2	4.8	not used
20 lb	3.4	not used	not used

* 1 accidental death.

A further series of tests on the effect of different dosage rates of toxaphene, strobane and thiodan on cased final instar larvae of *C. alcyonipennella?* were conducted during March 1958. Thiodan was included because, at that time, it was thought to be safe for use on crops visited by honey bees. Subsequent work (Palmer-Jones 1958; Palmer-Jones *et al* 1959) has shown that thiodan could cause heavy mortality to honey bees if applied to flowering crops. Five replicates of each dosage of insecticides were used, each with 5 larvae. The mean survivals following 48 hours exposure are shown in Table 3.

DISCUSSION

In the untreated portion of the field in which the toxaphene trials were conducted, the apparent reduction in seed yield due to *Coleophora* spp. was high considering the low population level of overwintering larvae. This may be an indication that infestation of a clover seed area by *Coleophora* spp. can occur by migration of moths from older adjacent clover pastures.

Palmer-Jones *et al* (1958) estimated the density of white clover flowers in the toxaphene treated area at 326,700 heads per acre. Using seed yields per 100 heads, the estimated yield per acre following application of toxaphene at 15 lb per acre was 42 lb against 8 lb on the untreated area. Although adequate provision was made for fertilisation of clover flowers, there being 34 hives of bees in the paddock, dry soil conditions and sparseness of clover flowers were responsible for the relatively low yield of seed on the treated area.

The validity of the seed yield results shown in Table 2 is subject to the limitations imposed by lack of replication and the possibility that the treatment could have introduced sampling bias. The results, however, are in line with the reduction in adult populations of *Coleophora* spp. as revealed by sweep sampling.

Owing to the limited sampling on the strobane treated areas no firm conclusions can be drawn on the effectiveness of this insecticide for the control of adults of *Coleophora* spp.

Laboratory experiments indicated that neither toxaphene nor strobane can be expected to provide good control of final instar larvae of *C. alcyonipennella?* The effect of these insecticides on earlier instar larvae has not been assessed. Now that it is known that toxaphene and strobane can be used on white clover crops in flower with little risk to honey bees further field trials of these insecticides against *Coleophora* spp. are warranted. Because of the limited acreage of white clover seed now harvested in Hawke's Bay, future trials should be conducted in Canterbury.

REFERENCES

Kelsey, J. M. 1958: Damage by Clover Case-bearer Caterpillars. *N.Z. J. agric. Res. 1:* 525–6.

Palmer-Jones, T. 1958: Laboratory Methods for Measuring the Toxicity of Pesticides to Honey Bees. *Ibid. 1:* 290–300.

Palmer-Jones, T.; Forster, I. W.; Line, L. J. S. 1958: Effect on Honey Bees of Toxaphene and Strobane Applied to White Clover Pasture. *Ibid. 1:* 694–706.

Palmer-Jones, T.; Forster, I. W.; Jeffery, G. L. 1959: Effect on Honey Bees of DDT and Thiodan Applied from the Air as Sprays to Chou Moellier. *Ibid. 2:* 481–7.

YEASTS FROM SOILS UNDER FOREST AND UNDER PASTURE

By M. E. di Menna, Soil Bureau, Department of Scientific and Industrial Research, Wellington

(*Received for publication, 12 May 1960*)

Summary

Two kinds of change were seen in the soil yeast flora when native broadleaf forest was replaced by introduced ryegrass – white-clover pasture. A yellow-brown loam under forest contained small numbers of *Candida curvata*; under pasture yeast numbers were greater and the population dominated by *Cryptococcus terreus*. In a recent gley soil under forest *C. curvata* was dominant but *Hansenula* spp. were also present in considerable numbers. In this soil under pasture yeast numbers were less than under forest; *Hansenula* spp. were absent and C. curvata was the sole dominant.

In a second yellow-brown loam, under pasture and subject to drought, *C. curvata, Cryptococcus albidus* and *Cr. terreus* occurred in low numbers and approximately equal proportions together with many contaminant yeasts from pasture plant leaves.

A yellow-grey earth under pure perennial ryegrass pasture supported a yeast flora similar to that in the recent gley soil under pasture but in greater numbers.

Introduction

In previous studies (di Menna 1958, 1960) yeasts of tussock grassland soils were examined under original vegetation and under introduced pasture plants. Change in kind of grassland had little effect upon the yeast flora. In the present work yeasts were studied in the A horizon of four soils which had once been under native forest but which had been cleared, cultivated and sown with introduced pasture plants. On two of the soils, Kiwitea silt loam near Marton and Gollans silt loam near Paraparaumu, sites under the native broadleaf-podocarp forest were available for study next to the sites under pasture. One site on a third soil, Tokomaru silt loam near Palmerston North, was under an experimental, single species pasture of perennial ryegrass (*Lolium perenne*), and a second site was under the more usual pasture mixture of perennial ryegrass and white clover (*Trifolium repens*). A fourth soil under pasture, Horotiu sandy loam at Claudelands Showgrounds in Hamilton, was studied as part of an investigation into the cause of facial eczema.

Kiwitea soil is classified as a yellow-brown loam; Gollans, as a recent gley soil; Horotiu, as a yellow-brown loam; and Tokomaru, as a yellow-grey earth.

The sites under pasture had been cleared of forest 50–80 years ago; the Tokomaru soil had been drained about 40 years ago. Stock were excluded from the two forested sites; the sites under pasture were grazed, principally by sheep. Annual rainfall at the sites varied between 39 and 46 in.

Chemical analyses of the soils are given in Table 1.

TABLE 1. CHEMICAL ANALYSIS OF SOILS STUDIED

	Kiwitea		Horotiu Pasture	Gollans		Tokomaru Pasture
	Forest	Pasture		Forest	Pasture	
pH	5.8	5.8	5.6 – 5.9	5.9 – 6.6	5.5 – 6.1	5.7
Total N %	0.96	0.65	0.32– 0.37	0.45– 0.49	0.37– 0.47	0.67
C/N	12	12	11 –12	10 –11	9 –12	14
Exchange capacity m.e.%	55.0	31.4	10.9 –12.7	11.6 –33.8	15.2 –17.1	14.7
Total exchang. bases m.e.%	44.0	16.3	8.3 –10.9	9.4 –15.5	4.6 – 6.4	7.4
% Base saturation	80	52	76 –86	39 –46	28 –42	48

Figures for Horotiu and Gollans soils are ranges given by three samples.

Figures for Tokomaru soil are abstracted from New Zealand Soil Bureau, 1954, except for Total Nitrogen and for Carbon/Nitrogen which are from Fife, 1945.

METHODS OF SAMPLING

The usual method of sampling was to take soil, with sterile precautions, at a depth of 2–3 in. from the side of a freshly dug pit. The Horotiu soil was sampled more shallowly, at a depth of $\frac{1}{2}$–$1\frac{1}{2}$ in. by means of a borer. On one occasion, so that it could be compared with the other soils in this work, it was also sampled at the 2–3 in. level. At each collection one sample was taken from each of five pits or bore holes spaced along a line about 100 yd long.

The Kiwitea soil under forest and under pasture was sampled on three occasions—in February, May and October, 1957. The Gollans soil under forest and under pasture was sampled once, in April, 1958. The Tokomaru soil was sampled under ryegrass in August and in December 1957, and under ryegrass-white clover in December 1957. The Horotiu soil was sampled on five occasions: in February, March, July, October 1957, and in January 1958. The 2–3 in. samples of Horotiu soil were taken in July.

Samples of leaves of pasture plants were taken from the sites under pasture on each occasion soil was collected and the yeasts on them examined. Results of this work have been published separately (di Menna 1959a). Samples of freshly fallen leaves and twigs from the L layer and of decomposing material and humus from the F + H layers were taken from the Kiwitea site under forest in February and in May.

METHODS OF CULTIVATION

Samples were cultured as soon as possible after collection, usually within twenty-four hours and always within forty-eight. From each sample 10g soil were weighed out with sterile precautions and made into serial dilutions of 1/250, 1/500 and 1/1,000 with sterile tapwater. Inocula of 0.5 ml were spread on the surfaces of 10 cm plates of acidified glucose peptone agar (glucose 4%, peptone 1%, brought to a pH of 4 by the addition of 10% HCl to the molten medium immediately

before the plates were poured). Three plates were inoculated from the 1/250 dilution of each sample, three from the 1/500 and four from the 1/1,000. Plates were incubated upside down at room temperature and counts were made after 4–6 days.

The forest litter samples collected from the Kiwitea soil in February were not cultured quantitatively, but the May samples were. Five g of each of the May samples were diluted serially to 1/50, 1/500, 1/1,000 and 1/10,000, and cultured as above.

Where possible twenty yeast colonies per soil or litter sample were picked from a plate or plates to give an optimum of 100 isolates per soil collection of five samples. The isolates were subcultured in glucose yeast extract broth of pH 4 to rid them of any bacterial contaminants, and then transferred to solid media of neutral pH for identification by

TABLE 2. YEASTS ISOLATED FROM THE KIWITEA SOIL (YELLOW-BROWN LOAM) UNDER NATIVE FOREST AND UNDER PASTURE
(Proportions of different species present are expressed as percentages)

	Forest			Av.	Pasture			Av.
	Feb.	May	Oct.		Feb.	May	Oct.	
Saccharomyces delbrueckii Lindner	4	—	—	1.3	—	—	-	--
Sporobolomyces roseus	2	—	—	0.7	—	—	—	--
Sporobolomyces odorus Derx	—	—	3	1	—	—	—	
Cryptococcus laurentii	2	3	7	4	1	4	3	2.7
Cryptococcus albidus	—	3	3	2	12	11	34	19
Cryptococcus terreus	2	1	3	2	48	77	53	59.3
Cryptococcus gastricus Reiersol et di Menna	–	1	—	0.3	—	–	—	—
Torulopsis ingeniosa	—	—	—	—	6	1	—	2.3
Candida humicola	—	1	1	0.7	—	—	—	-
Candida parapsilosis (Ashf.) Langeron et Talice	—	1	—	0.3	—	—	-	—
Candida curvata	69	80	67	72	7	6	4	5.7
Trichosporon cutaneum	10	9	15	11.3	4	1	6	3.7
Rhodotorula glutinis	5	1	—	2	–	–	—	-
Rhodotorula mucilaginosa (Jorg.) Harrison	—	—	—	—	1	—	—	0.3
Rhodotorula minuta (Saito) Harrison	2	—	—	0.7	—	–	—	·
Rhodotorula graminis	—	—	—	—	18	—	—	6
Pullularia pullulans	2	–	—	0.7	—	—	—	—
Unknown	2	—	1	1	3	—	—	1
NUMBER YEASTS ISOLATED	55	68	74	—	100	100	100	—
Av. count/g in thousands	2.0	1.4	3.0		9.6	9.6	36.6	
Range of count/g in thousands	1–3	1–2	1–8		6–18	4–19	24–50	

the criteria of Lodder and Kreger-van Rij (1952). *Pullularia pullulans* (De Bary et Low) Berkhout was treated as a yeast.

Results

Results are given in Tables 2, 3, 4, and 5.

TABLE 3. Yeasts Isolated from the Horotiu Soil (Yellow-brown Loam) Under Pasture
(Proportions of different species present are expressed as percentages)

	At ½–1½ in.					Av.	At 2–3 in.
	Feb.	Mar.	Jul.	Oct.	Jan.		Jul.
Debaryomyces kloeckeri Guill. et Peju	—	1	2	—		0.6	—
Debaryomyces nicotianae (Zach) Loder et van Rij	—	—	—	2	—	0.4	—
Sporobolomyces roseus	21	6	—	—	—	5.4	—
Bullera alba (Hanna) Derx	—	1	—	5	—	1.2	—
Cryptococcus laurentii	7	17	13	18	55	22	3
Cryptococcus albidus	—	1	9	22	13	9	35
Cryptococcus terreus	1	2	8	15	13	7.8	25
Cryptococcus gastricus	—	—	1	—	—	0.2	—
Torulopsis candida (Saito) Lodder	—	—	1	—	—	0.2	—
Torulopsis famata (Harr.) Lodder et van Rij	—	—	—	—	—	—	2
Torulopsis inconspicua Lodder et van Rij	—	—	—	2	—	0.4	—
Torulopsis ingeniosa	1	3	28	10	5	9.4	—
Candida humicola	—	1	—	—	—	0.2	—
Candida rugosa (Anderson) Diddens et Lodder	—	—	—	1	—	0.2	—
Candida curvata	5	1	19	10	2	7.4	27
Trichosporon cutaneum	—	—	—	—	2	0.4	—
Rhodotorula glutinis	29	19	5	1	—	10.8	—
Rhodotorula mucilaginosa	1	3	1	6	—	2.2	5
Rhodotorula flava (Saito) Lodder	—	—	1	2	—	0.6	—
Rhodotorula marina Phaff, Mrak et Williams	—	—	1	—	6	1.4	—
Rhodotorula crocea Shifrine et Phaff	—	—	1	—	—	0.2	—
Rhodotorula texensis Phaff, Mrak et Williams	—	—	—	1	—	0.2	—
Rhodotorula graminis	33	37	8	—	2	16	3
Pullularia pullulans	2	—	—	—	—	0.4	
Unknown	—	8	2	5	2	3.4	—
NUMBER YEASTS ISOLATED	87	97	85	95	62	—	60
Av. count/g in thousands	7.2	14.4	6.0	6.2	2.4		4.8
Range of count/g in thousands	2–17	3–36	1–10	4–10	1–5		1–16

Candida curvata (Diddens et Lodder) Lodder et van Rij was dominant in the Kiwitea soil under forest; in the same soil under pasture *Cryptococcus terreus* di Menna was dominant and together with *Cryptococcus albidus* (Saito) Skinner made up 78% of the isolates. Numbers of yeasts were consistently low in the forested soil, from 1,000 to 8,000/g of soil (average 2,100), but were higher in the soil under pasture, from 4,000 to 50,000/g (average 18,600).

TABLE 4. YEASTS ISOLATED FROM THE GOLLINS AND THE TOKOMARU SOILS
(Proportions of different species present are expressed as percentages)

	Gollans		Tokomaru			
	April		August	December		Av.
	Forest	Pasture	Ryegrass	Ryegrass	Ryegrass + Clover	
Saccharomyces delbrueckii	1	—	-	-	—	- -
Hansenula californica	17	—	—	—	—	—
Hansenula mrakii	23	—	—		—	—
Hansenula canadensis	—	—	1		-	0.3
Debaryomyces kloeckeri	—		1		-	0.3
Cryptococcus laurentii	3	3	1	3	1	1.7
Cryptococcus albidus	2	11	19	11	—	10
Cryptococcus terreus	1	1	—	2	—	0.7
Torulopsis ingeniosa	—	—	2		1	1
Candida pulcherrima (Lindner) Windisch	1	—	—	—	—	—
Candida tropicalis (Cast.) Berkhout	1	—	—	—	—	—
Candida humicola	2	—	1	—	—	0.3
Candida curvata	47	63	65	82	97	81.3
Candida bovina	—	7	—	—	—	—
Trichosporon cutaneum	1	13	7	—	1	2.7
Schizoblastosporion starkeyi-henricii Ciferri	—	1	—	—	—	
Rhodotorula mucilaginosa	—	—	1			0.3
Rhodotorula graminis	—	1	—	1	—	0.3
Unknown	1	—	2	1	—	1
NUMBER YEASTS ISOLATED	100	91	85	100	91	—
Av. count/g in thousands	15.4	6.4	7.4	31.8	21.4	
Range of count/g in thousands	6–30	2–14	2–14	16–79	6–38	

In the Horotiu soil under pasture, from a depth of 2–3 in., there were approximately equal numbers of *C. curvata, Cr. albidus* and *Cr. terreus*. The Horotiu soil from $\frac{1}{2}$ $1\frac{1}{2}$ in. also contained these species in similar proportions, but in addition contained large numbers of contaminant species from the leaves of pasture planes, viz. *Sporobolomyces roseus* Kluyver et van Niel, *Cryptococcus laurentii* (Kufferath) Skinner,

TABLE 5. Yeasts Isolated from Litter from the Kiwitea Soil Under Forest

	L Layer		F + H Layer	
	February	May	February	May
Saccharomyces pastori (Guill.) Lodder et van Rij	—	—	—	1
Debaryomyces subglobosus (Zach) Lodder et van Rij	1	—	—	—
Sporobolomyces roseus	—	1	—	—
Cryptococcus laurentii	3	14	—	—
Cryptococcus luteolus (Saito) Skinner	—	1	—	—
Torulopsis glabrata (Anderson) Lodder et de Vries	—	—	1	—
Torulopsis ingeniosa	2	—	—	1
Candida humicola	3	2	12	11
Candida rugosa	1	—	1	—
Candida scottii Diddens et Lodder	3	—	—	—
Candida curvata	2	—	—	—
Trichosporon cutaneum	—	2	4	6
Rhodotorula glutinis	2	—	1	—
Rhodotorula mucilaginosa	1	—	—	—
Rhodotorula graminis	2	—	—	—
Pullularia pullulans	—	20	1	1
TOTAL	20	—	20	20
Yeasts/g in thousands	—	40	—	4

Torulopsis ingeniosa di Menna, *Rhodotorula glutinis* (Fres.) Harrison, *Rhodotorula graminis* di Menna, and other *Rhodotorula* spp. These leaf species did not appear to be able to multiply or maintain their numbers in the soil; species most common in the soil were those most common on leaves at the time the soil was collected. Yeasts on leaves of pasture plants have been shown to vary with season, *Cr. laurentii* and *T. ingeniosa* being dominant in winter and spring, the red pigmented *Sporobolomyces* and *Rhodotorula* spp. in the summer and early autumn (di Menna 1959a). Counts in Horotiu soil were usually low. At the 2–3 in. level they ranged between 1,000 and 16,000/g. At $\frac{1}{2}$–$1\frac{1}{2}$ in. they were sometimes higher, but 43-92% of the population consisted of leaf species.

In the Gollans soil under forest *C. curvata* made up 47% of the isolates; a further 40% consisted of two fermenting species, *Hansenula californica* (Lodder) Wickerham and *Hansenula mrakii* Wickerham. Neither of these *Hansenula* spp. were isolated from Gollans soil under pasture, in which *C. curvata* made up 63% of the isolates. Counts in forested Gollans soil were higher than counts in Gollans soil under pasture; in the soil under forest there were from 6,000 to 30,000 yeasts/g (average 15,400), under pasture from 2,000 to 14,000 (average 6,400).

C. curvata was the dominant yeast in Tokomaru soil, both under pure ryegrass pasture (74% of isolates) and under ryegrass – white clover (97%). Yeast counts in this soil varied between 2,000 and 79,000/g.

In the three soils sampled more than once, Kiwitea, Horotiu and Tokomaru, counts were greatest during the warmer months of the year, from October to March, but the increments were never large. Almost no seasonal effect could be seen in the Kiwitea soil under forest.

No one species was dominant in the sample from the L layer collected from the Kiwitea soil under forest in February; *Cr. laurentii* made up 14 of the 20 isolates from the May collection. *Candida humicola* (Daszewska) Diddens et Lodder was dominant in both the February and May collections of F + H layer.

DISCUSSION

Two kinds of change were seen in the soil yeast flora when forest was replaced by pasture. In Kiwitea soil proportions of *Cr. terreus* and *Cr. albidus* and total numbers increased whilst proportions of *C. curvata* diminished greatly. Dominant yeasts of soil under native tussock grassland have been shown (di Menna 1958, 1960) to be the nitrate assimilating *Cryptococcus* spp., *Cr. Albidus*, *Cr. terreus* and *Cryptococcus diffluens* (Zach) Lodder et van Rij, so this change is to be expected. The same sort of change appears to have occurred in Horotiu soil but not to have gone so far, for *C. curvata*, *Cr. Albidus* and *Cr. terreus* were isolated in equal proportions. This lag in conversion from species typical of forest to those typical of pasture may be due to decimation of the soil yeast flora by heat and desiccation. Summer temperatures in Horotiu soil are higher than those in the more southerly Kiwitea soil, and the sandy nature of the Horotiu soil gives it less moisture holding capacity and makes it liable to drought (N.Z. Soil Bureau 1954, p. 246). Heat and desiccation disturbing the soil flora as a whole may also account for the invasion of Horotiu soil by leaf yeasts. There were 58% leaf species in this soil collected from the $\frac{1}{2}$–$1\frac{1}{2}$ in. level in July, 11% in soil from 2–3 in. taken at the same time. Proportions of leaf species in Taita silt loam under pasture, which has slow subsoil drainage, collected in the Hutt Valley in May, were 13% at $\frac{1}{2}$–$1\frac{1}{2}$ in. and 2% at 2–3 in.

The change in the yeast flora of the Gollans soil following conversion of forest to pasture was different to that in Kiwitea soil, both quantitatively and qualitatively. The soil under forest contained, as well as *C. curvata*, fermenting *Hansenula* spp. as subdominants. Change to pasture resulted in decrease of total yeast numbers, disappearance of *Hansenula* spp., and increase in proportions of *C. curvata*. Proportions of *Cr. albidus* altered from 2% in forest soil to 11% in pasture soil, an alteration towards the natural grassland flora, but one which had proceeded only a small way.

In Tokomaru soil under pasture the species pattern was very much the same as in Gollans soil under pasture. As one isolate of *Hansenula canadensis* Wickerham was recovered from Tokomaru soil it is tempting to suggest that this was a remnant of the Tokomaru soil flora under

forest, which like the Gollans soil under forest contained a proportion of *Hansenula* spp. However it is unsafe to attach much significance to single isolates. Although limited numbers of taxa are recovered from soils by the methods used in this work, it is probably possible, by means of suitable techniques, to recover yeasts of all described genera and of very many species from one soil. The effect of isolation temperature alone upon the kinds of yeasts recovered from soil has been shown in earlier work (di Menna 1955).

This is the first occasion upon which *Hansenula* spp. have been shown to be common in a New Zealand soil, although members of the genus have been found in soils in many parts of the world. Disregarding its fermenting powers, the genus *Hansenula* possesses a number of the characteristics considered to be typical of soil yeasts (di Menna 1955, 1959b): use of nitrate nitrogen, ability to use a variety of compounds as carbon sources, and, although not invariably, independence of an external source of growth factors (Wickerham 1951).

Factors affecting the distribution of organisms can be conveniently, if arbitrarily, divided into physico-chemical, nutritional and antibiotic. A physico-chemical factor soil moisture or a factor consequent upon it —may have played a part in determining pattern of yeasts in the four soils studied.: A characteristic common to Gollans and Tokomaru soils is poor drainage; Kiwitea and Horotiu soils are both well drained. It seems possible that the higher moisture content of the Gollans and Tokomaru soils, directly or indirectly, has retarded the change from the *C. curvata* dominated population of forested soil to a *Cryptococcus* dominated population typical of grassland soil, or even halted it altogether. At the other extreme, good subsoil drainage and poor moisture retaining capacity in Horotiu soil together with rather high summer temperatures, have modified the yeast flora by killing much of it.

The effects of nutrients can be seen in the distribution of *Candida humicola*. This yeast is pectinolytic (di Menna 1959b) and has been found in large numbers only where decaying vegetation is abundant, in the root zone of tussock plants (di Menna 1958, 1960), in peat (Wright 1959, p. 27) and in forest litter.

Antibiotic effects can only be speculated upon at this point. It is possible that nutrional and physico-chemical effects may not act directly upon the soil yeast flora, but upon other antibiotic producing organisms which regulate the yeast population. A substance toxic to *Cryptococcus* spp. but not to *C. curvata* has been demonstrated in an autoclaved soil extract (di Menna 1957), and it was wondered whether the differences between the yeast flora of the Kiwitea soil under forest and under pasture might be due to the presence of a similar substance in forested soil. A soil extract agar was made from Kiwitea soil under forest (method in di Menna 1957) and three samples of Kiwitea soil under pasture were cultured on it and in parallel on glucose peptone agar in the standard way. Results are given in Table 6. Differences between proportions of *Cr. albidus, Cr. terreus* and *C. curvata* recovered on the two media were not great and seemed due to chance. It was curious that the soil extract medium appeared to inhibit *Trichosporon cutaneum* (de Beurm.,

TABLE 6. EFFECT OF FOREST SOIL EXTRACT UPON YEASTS ISOLATED FROM THE KIWITEA SOIL UNDER PASTURE

	Sample 1		Sample 2		Sample 3		Total	
	GPA	FSA	GPA	FSA	GPA	FSA	GPA	FSA
Cryptococcus laurentii	—	—	1	—	—	—	1	—
Cryptococcus albidus	5	5	6	5	11	11	22	21
Cryptococcus terreus	11	13	7	10	4	7	22	30
Torulopsis ingeniosa	—	—	3	2	—	—	3	2
Candida curvata	—	2	—	3	3	2	3	7
Trichosporon cutaneum	4	—	3	—	2	—	9	—
TOTAL	20	20	20	20	20	20	60	60
Yeasts/g in thousands	12	14	21	18	21	12	—	—

GPA: Glucose peptone agar used as isolation medium.
FSA: Soil extract agar from Kiwitea soil under forest used as isolation medium.

Gougerot et Vaucher) Ota which made up 11% of the total isolates from the Kiwitea soil under forest and only 3% of isolates from the same soil under pasture. In Gollans soil under pasture however there was a greater proportion of *Tr. cutaneum* (13%) than in the same soil under forest (1%).

The distribution of *Tr. cutaneum* is rather odd. It has been found in small proportions in many of the New Zealand soils so far examined, but has not yet been found as a dominant. Van Uden, Do Carmo Sousa and Farinha (1958) isolated it regularly from the caeca of horses, but its regular incidence in soil suggests that, unlike *Torulopsis glabrata* (Anderson) Lodder et de Vries and *Candida bovina* van Uden et Do Carmo Sousa, which are obligate saprophytes of animals and occur only sporadically in soil, it is able to occupy both ecological niches.

ACKNOWLEDGMENTS

My thanks are due to Dr A. T. Johns, Plant Chemistry Division, Department of Scientific and Industrial Research, for permission to sample Tokomaru soil under experimental pasture; to Mr H. S. Gibbs and Mr A. P. Druce, Soil Bureau, who assisted in the selection of sites; and to Miss F. A. D'Ath, Mr M. Fitzpatrick and Mr W. R. Owers, Soil Bureau, who made the chemical analyses of the Kiwitea, Gollans and Horotiu soils.

REFERENCES

Fife, C. V. 1945: Study of a Yellow-Grey Loam in the Manawatu. *N.Z. J. Sci. Tech. A26:* 281–93.

Lodder, J.; Kreger-Van Rij, N. J. W. 1952: "The Yeasts: A Taxonomic Survey." North-Holland Publishing Co., Amsterdam.

Menna, M. E. di 1955: A Search for Pathogenic Species of Yeasts in New Zealand Soils. *J. gen. Microbiol. 12:* 54.

————1957: The Isolation of Yeasts from Soil. *Ibid. 17:* 678.

————1958: Biological Studies of Some Tussock Grassland Soils. III. Yeasts. *N.Z. J. agric. Res. 1:* 939.

————1959a: Yeasts from the Leaves of Pasture Plants. *Ibid. 2:* 394.

———1959b: Some Physiological Characters of Yeasts from Soils of Allied Habitats. *J. gen. Microbiol. 20:* 13.
———1960: Biological Studies of Some Tussock Grassland Soils. XIV. Yeasts of Two Cultivated Soils. *N.Z. J. agric. Res. 3:* 207-13.
N.Z. Soil Bureau 1954: "General Survey of the Soils of the North Island, New Zealand." *N.Z. Soil Bur. Bull. 5.*
Uden, N. Van; Carmo Sousa, L. Do; Farinha, M. 1958: On the Intestinal Yeast Flora of Horses, Sheep, Goats and Swine. *J. gen. Microbiol. 19:* 435.
Wickerham, L. J. 1951: Taxonomy of Yeasts. *Tech. Bull. U.S. Dep. Agric. 1029.*
Wright, A. C. S. 1959: Soils of Chatham Island. *N.Z. Soil Bur. Bull. 19.*

DEVELOPMENT OF A METHOD FOR ROUTINE TESTING OF POULTRY FEEDS

By Jean G. Miller, Principal Scientific Officer (Biometrics), and J. Edmondson, Poultry Experimentalist, Department of Agriculture

(*Received for publication, 4 April 1960*)

Summary

A description is given of a series of studies undertaken at the Department of Agriculture's Poultry Demonstration Plant to evolve an optimum test procedure for the nutritional value of commercial poultry feeds.

It shows how a logical sequence of small trials, each designed on information obtained in the preceding one, can economically provide the estimates of means and variances required in setting up a suitable test procedure which will have its probability of error below a given level.

The programme has been completed for chick feeds, and the preliminary work for a test on feeds for adult birds is also described.

Introduction

In developing a routine test on which commercial products could be given a grading, it is necessary to determine the variances attachable to treatment means so that a given level of confidence may attach to any results quoted. The variance of test results will usually arise from several sources and the most economic design of the test procedure will depend on the relative sizes of these separate components of variance. It is desirable to set a fixed level of performance in the routine test, below which a product will immediately be judged sub-standard, without computing a full statistical analysis for each trial. This is justifiable if the variance is known to be constant. The series of studies described here shows how the variances were estimated at the same time as practical experience was gained in testing commercial poultry mashes by their effect on the growth rate of very young chickens.

There are two main classes of feeds concerned—those suitable for growing chicks and those on which laying birds are fed. It is hoped that the growth of the very young chickens will be a reliable indicator of quality in both types. The trials reported here have led to a suitable test for chick feeds, and the work on laying mashes is still proceeding.

Equipment Used

A set of six cages, each to contain about 25 chicks, was set up in a 14 ft by 16 ft room of an open fronted "lean to" type poultry house.

Each cage is an individual unit set on a 27-in. high stand and measures 22 x 35 x $10\frac{1}{2}$ in. high with a small electric heater suspended from the top of the cage. Movable food and water troughs are fixed outside the cage and are raised as the chicks grow.

After the first test in which seven out of the eight deaths were the result of cannibalism, steps were taken to prevent direct sunlight falling on the cages and improvements were made in ventilation. Cannibalism occurred again in the second test but was less severe and with no fatality. In all further tests a routine of debeaking (removal of the tip of the upper mandible) was adopted at five days of age.

Chicks for the test are produced from cross-bred pullets, which are housed in single bird laying cages, and which are artificially inseminated using pooled semen from a team of 12 males.

Analysis of the weight gains in the early trials and consideration of practical aspects of management and procedure gave no reason for changing the design or size of cages and further sets have since been ordered and put into operation.

HISTORY OF THE TRIALS

General Technique

The first two trials were run to sort out points of practical technique including weighings. In the first, 28 birds per cage were used and these were weighed in bulk at 5, 21, and 26 days of age, one weight being obtained from each cage at each time. There was no apparent effect from position of cage in the room although the degrees of freedom were too few to test this with much sensitivity. A similar result was, however, obtained in the repeat test three weeks later. Mean weights and standard errors for the first test were:—

Weights per chick (in oz)

	At 5 days	At 21 days	At 26 days	Gain 5 to 21 days	Gain 5 to 26 days
Mean	2.15	6.41	7.90	4.26	5.75
S.E.	.062	.155	.115	.184	.144
Coeff. of Var.	2.9%	2.4%	1.5%	4.3%	2.5%

The coefficients of variation all show satisfactorily low levels. Although based on only 5 degrees of freedom, they were virtually confirmed in the repeat trial. These results from the two trials indicated that it mattered little statistically which day was chosen for the final weighing or whether final weight or weight gain was used as the test variable.

It was observed both here and in other work that the main mortality of chicks occurs in the first few days of life and that by the fifth day the numbers have stabilised. After results of the first trial, which had been started on the fifth day, were available, it was decided that little would be gained and probably much lost by attempting to have the trials begin earlier than the fifth day. For all trials then the chicks were all fed only coarse ground grain, thereby reducing the food nutrient reserves carried through from the maternal diet, from hatching until the fifth day when undersized and weakly chicks were rejected, the others allocated to cages and the test foods introduced.

After the cannibalism problem had been overcome there were very few deaths during the trial periods. This indicates that with this technique, using proper precautions, there should be no major problem of estimating treatment effects with complex adjustments because of unequal numbers surviving.

It is desirable that in large-scale testing, a routine pattern should be established in the sequence of operations on all tests. Hence if the tests begin at 5 days after hatching, it will be more convenient if they finish at 26 days, just 3 weeks later. All weighings on test chicks can then be confined to one given day of the week. The results of the first tests showed that a variate "weight gain from 5 to 26 days" was statistically acceptable, and as it was the most practically convenient, it was chosen for all further trials.

Variations per Cage and per Chicken

Nothing was known of the variability of individual chickens or of any differences between cages. It was necessary to estimate these to determine the number of chickens and relative positions of cages to be used in the final feed-testing scheme. Hence in the next trials of the series all chickens were wing banded and weighed individually, their sex also being recorded. The analysis of variance for weight gains of the 147 surviving birds was:

Variation	D.F.	Mean Square
Between cages	5	445
Sex	1	1117
Within cages	140	1105
Mean weight gain	151 g	
S.E. per cage mean	6.6 g	
C.V.	4.4%	

From this trial on all weights are in grammes.

This shows no suggestion of any "cage effect", or sex effect, while the coefficient of variation between cage means is very similar to that of the first two trials.

These two factors of sex, and grouping into cages may then be ignored and all that need be considered in the final design is the total number of birds required to give the desired accuracy. With the errors obtained so far and the differences likely to occur, it seems that fifty birds per treatment would be adequate. This will be considered in more detail when "probability of error" levels are finally fixed.

Sensitivity to Different Feeds

In all the trials so far the chicks had been fed the control chick mash as used at the Department of Agriculture's Poultry Demonstration Plant. In the next trial two cages were given this standard mash and the other four were given mash very low in protein. There was no doubt that the technique was able to demonstrate the effect of the lack of protein.

Details of all mashes fed in these trials are given in Table 1.

TABLE 1. DETAILS OF THE RATIONS USED IN THIS SERIES OF TRIALS
(expressed as percentages)

	1 Control Chick Mash	2 Test Mash	3 Different source of Meat Meal	4 Basic Laying Mash	5 Layers Mash, no Vitamin A added	6 Layers Mash, low Protein	7 Laying Cage Mash
Bran	5	5	5	5	5	5	3
Pollard	10	10	10	10	10	10	3½
Barley Meal	—	—	—	25½	25½	25½	15
Wheat Meal	50	50	50	30	34	36	44
Maize Meal	10	10	10	10	10	10	10
Meat Meal	12	3	12	8	8	5	11
Bone Meal	—	3	Samples (2)	—	—	—	—
Milk Powder	6	—	6	3	3	—	4
Oyster shell dust	1½	1½	1½	2	2	2	3
Salt	½	½	½	½	½	½	½
Vitamin A, D3, B2 premix	1	1	1	2	—	2	2
Vitamin D, B2 premix	—	—	—	—	2	—	—
Lucerne Meal	4	4	4	4	—	4	4
Manganese Sulphate	4oz/ton	4oz/ton	4oz/ton	4oz/ton	4oz/ton	4oz/ton	4oz/ton

Weight gain on control mash	201 g	Ration (1)
Weight gain on control test mash	79 g	Ration (2)

Individual weights were kept in this trial and the standard errors of the two groups were:

	S.E. per cage mean	C.V.
Ration 1—Control Chick Mash	8.4 g	4.2%
Ration 2—Test Mash	5.0 g	6.3%

The difference in these standard errors is significant, each being measured with quite a large number of degrees of freedom although the ratio is only 1.7. The difference in food values of these two mashes is much larger than any which the routine procedures will have to test. From the individual weights in this trial the previous result was confirmed, that there is no "between cage" variance.

For the next trial, two mashes were compared which differed only in the source of the meatmeal used. This was considered typical of the differences one might meet in commercial testing. Three cages each of 18 birds were fed each mash (there were insufficient birds available from the hatching for higher numbers). The whole trial was then repeated using 25 birds per cage.

	Mean Wt Gain (g)	
	1st Trial	2nd Trial
Ration 3—Using Meatmeal Sample 2	131	136
Ration 1—Control Chick Mash	179	157
Difference	48**	21*
S.E.	9.5	8.2
C.V.	6.1%	5.6%

The technique was again sufficiently good to show the difference in the feeds, even assuming that the error variance was based on only 4 degree of freedom. The magnitude of the difference is however significantly greater in the first than in the second trial.

At this stage it was decided that the technique had been shown to be suitable for comparing different feeds.

Results with Laying Mashes

If the chicks are also to be used as assay animals in the assessment of laying mashes, it is necessary to find out: (a) whether they will make reasonable growth on a laying mash; and (b) whether they will satisfactorily reflect differences in laying mashes. This requires running parallel tests on laying birds and chickens with the same mashes. Two pairs of trials have begun and the chick part completed, which latter is considered here.

The first pair of trials had three treatments each applied to two cages of 25 birds. Weight gains and standard errors were:

	Weight gain (g)	
	1st Trial	2nd Trial
Ration 1—Control chick mash	154 a	175 a
Ration 4—Basic laying mash	128 b	154 b
Ration 5—Laying mash, no vitamin A added	103 c	142 b
S.E.	4.3	6.0
C.V.	3.3%	3.8%

The chickens grew reasonably well on the laying mash but not as well as on the special chick mash. The second test failed to show a difference due to the omission of vitamin A which the first test had shown, although the interaction of treatments x trials was not significant, i.e., the depression of 25 g in the first trial was not significantly different from the depression of 12 g in the second.

The second pair of trials again had three treatments:

	Weight gain (g)	
	1st Trial	2nd Trial
Ration 4—Basic laying mash	154 a	150 a
Ration 6—Layers mash with low protein	79 b	86 b
Ration 7—Laying cage mash	145 a	144 a
S.E. (rations 4 and 7 only)	6.2	9.9
C.V. (rations 4 and 7 only)	4.1%	26.8%

These two trials gave very similar results. The laying mashes used in these last four trials are all being tested over several months on adult birds with individual egg records being kept. The relationship of these tests to the chick trials will be reported when the whole phase of the programme has been completed. Results of these trials have been included here as they give extra estimates of the variance of the chicks.

Estimation of a Pooled Error

The standard errors per chick obtained in all the trials are shown in the following table, along with the mean weight gain and the coefficient of variation of the cage means (there were different numbers of birds in the different trials):

Mean weight gain (g)	S.E. per chick	C.V. of cage Means
160	20.9	2.6%
174	43.5	5.0%
151	32.9	4.4%
201	33.2	4.2%
79	20.3	6.3%
155	40.3	6.1%
146	41.0	5.6%
128	41.7	3.3%
157	29.8	3.8%
150	30.3	4.1%
146	54.3	6.8%

From a sketch graph there did not appear to be a dependence of standard error on mean weight gain, and the errors all fell within a reasonably small range. (They did not entirely satisfy the Bartlett test for homogeneity, but this test can be more sensitive to lack of normality than to heteroscedasticity). For the purposes of this project it seems reasonable to pool the estimates, as it appears that an acceptable protection level can be used without an impractically large number of chickens, for any of the variances here obtained.

The pooled variance per cage of 25 chicks is 53.6 g, with S.E 7.3 g, in units of mean weight gain per chick. Using this estimate and considering it to be based on a very large number of degrees of freedom, we find that the differences in grammes per chicken required for significance are:

	5%	1%
2 cages of 25 chicks per treatment	14.3	18.8
3 cages of 25 chicks per treatment	11.8	15.5

If it is considered unjustifiable to use a large number of degrees of freedom, the significant differences calculated on only the degrees of freedom available between cages, are:

	5%	1%
2 cages of 25 chicks per treatment	23.4	42.3
3 cages of 25 chicks per treatment	16.8	27.6

It happens that in the trials conducted so far almost all treatment differences would have been either significant on both tests or on neither.

Food Consumption During the Tests

Details were kept of the amount of food eaten in each cage in all the trials (sufficient food was always kept before the birds). It was clear that some of the feeds were markedly unpalatable and weight gains on these were very poor. There was, however, no relation between the weight gained and the amount eaten in replicate cages on the same feed. No adjustments therefore were made to weight gains because of differences in consumption. There was no instance of a mash showing weight gains equal to the standard on a smaller food consumption but evidence of this will be watched for in commercial testing.

Design of a Test

The variance of the result of a test conducted with this technique and using any given number of birds can now be reasonably estimated. Hence when the requirements of a "satisfactory" commercial feed have been decided it will be possible to specify the number of cages required. If for instance a "satisfactory" mash is one which "has a performance not worse than the standard" then a suitable test procedure could be to use 3 cages of chickens per treatment and call a mash unsatisfactory if the average weight gain of the test chickens was below that of the control chickens by more than 15 g. Such a system would have a probability of only 1% of rejecting a mash which was actually as good as the standard. On the other hand there would be a probability of 50% of classing as satisfactory a mash which was truly inferior to the standard by just exactly 15 g. Mashes with a true value below the standard but closer to it than 15 g would have more than a 50% chance of being considered satisfactory on their test results.

Before deciding these "satisfactory" levels, and the acceptable probabilities of making a mistake in any one case, a series of tests will now be run in which a wide range of feeds will be compared and their general "spread" studied.

Drawing the Sample for the Test

The probabilities of error quoted above refer not to the true value of the whole bulk of a commercial line, but only to the variabilities of results of a test on the sample, introduced by the different chickens which are used in the assay. In commercial testing, retests will be possible and calculation of probabilities will have to take this into account. Also work will have to be done on the variability of samples taken from the bulk before details of the frequency of testing and re-testing can be specified.

Discussion

With the completion of the current series of trials in which a wide range of meatmeals will be tested, and any differences examined between the extra rooms in the poultry house which have now been equipped with cages, sufficient information will be available for the establishment of a routine testing scheme, if this should prove desirable. It seems likely that, for every test, a simple inspection of the average weight gains per chick will be sufficient for assessing the feed without the necessity of a full statistical analysis. By setting a limit to the range of variation tolerable between the means for the separate cages of any one feed, it will be possible to ensure that the assumptions on variances made in the setting of the inspection criteria are being justified. Where the cage means fall outside the tolerance, the full data of the test can be examined, and, if necessary, the test repeated.

WINTER GROWTH OF LUCERNE (*Medicago sativa*)

II. GENETIC VARIATION IN A POPULATION OF HUNTER RIVER LUCERNE

By T. P. Palmer, Crop Research Division, Department of Scientific and Industrial Research, Christchurch

(*Received for publication, 12 April 1960*)

Summary

Genetic variation in winter growth was estimated in 98 unselected Hunter River plants, and in the polycross progeny of 85 of them. The lengths of the longest stems of the parent plants and their progenies were measured at intervals late in the winter of 1958, and then the plants were cut and weighed.

Estimates of the genetic proportion of the variation in length and green weight as derived from the analysis of variance of the data from parents and offspring, or from the covariance of parenst and offspring, were high.

Selection for mean length based on parental phenotypes was as effective as selection on progeny performance, but phenotypic selection for green weight was not effective.

There was a small negative correlation between initial lengths and growth increments during the measurement period in the whole population, but when the population was divided into upper and lower fractions of different final stem lengths, these two characters were strongly and negatively correlated in both fractions.

Introduction

There is considerable variation in the amount of winter growth of different lucerne varieties (Zaleski 1954; Morley *et al.* 1957; Palmer 1959). When lucerne plants are grown at wide spacings, considerable differences between plants can be detected. If these differences are transmitted from parent to offspring it should be possible to increase the winter growth of a variety by selection within it. The success of selection depends largely on the ratio of the genetic to the total variation of the character in the population, the heritability.

There are broadly two methods of estimating the heritability in cross-fertilised perennials which can be vegetatively propagated:

(1) Estimates can be based on analysis of the variation between and within clones. The within-clone variance gives a measure of environmental variation, and the between-clone variance an estimate of the genetic variation.

(2) Heritabilities can be estimated from differences between progenies and from parent-offspring relationships derived from progeny tests, which may be of several sorts depending on the male parent or parents used.

Differences between clones give only an estimate of the genetic differences between plants. They give no indication of how these

differences are transmitted. In so far as differences are caused by dominance or epistatic effects, estimates derived from clones will be higher than estimates derived from progeny tests. Where a common male parent is used to produce progenies, differences between them will be lower.

The utility of the different methods of progeny testing may well vary from population to population. In general, these tests are used to detect parents which, when crossed together, will give progeny with better performance than average. In essence the selection process aims at isolating a maximum number of the high-performance genes in a minimum number of parents, so producing a population with a maximum concentration of such genes. This may require several cycles of selection.

Progeny testing is relatively expensive, and where the heritabilities of the traits being selected are high, it may with advantage be preceded by selection based on the phenotypes of the original population. In fact, such phenotypic selection is usual, even when heritabilities are low.

In this paper the results of a study of the inheritance of winter growth in a population of unselected Hunter River lucerne plants are reported.

Material and Methods

Clones from 100 Hunter River lucerne plants were grown from stem cuttings and planted at Lincoln in the spring of 1956 in a randomised block polycross nursery of six replications, with plants 75 cm apart. One plant survived in only one replication, and another in two; these have been omitted from the analysis. Seed was harvested in the autumn, and the progeny planted in the spring of 1957. The seed yield from the polycross nursery was low and only 85 plants gave enough seed for three replications in the progeny test. Plots in the progeny test consisted of a sngle row of 10 plants, spaced 75 cm apart.

The length of the longest stem on each plant in the polycross nursery and progeny blocks was measured on 14 and 29 August and 11 and 23 September 1958. Between 1 and 3 October, each plant in the polycross nursery and each plot in the progeny blocks was cut and weighed.

Symbols

M_L = mean square between lines
M_E = error mean square
b = number of blocks
cov(PO) = covariance of parent and offspring means

Result and Discussion

Heritabilities and Additive Genetic Variance

Heritability estimates derived from the analysis of variance of the parent clones by the formula $h^2 = \frac{M_L - M_E}{M_L + (b-1)M_E}$ are given in Table 1.

Following Comstock (1952), quoted by Kehr and Gardner (1960), the genetic variance between parent clones is given by the formula $\frac{M_L - M_E}{b}$, and estimates of the additive portions of this are provided from offspring data by $^1/b\ (M_L - M_E)$ and from parent offspring data by 2 cov(PO). These estimates are also given in Table 1.

TABLE 1. HERITABILITY ESTIMATES AND GENETIC COMPONENTS OF VARIANCE

Based on	Heritability Estimates				
	Stem Lengths				Green Weight
Parents	14 Aug	29 Aug	11 Sep	23 Sep	
$h^2 = \frac{M_L - M_E}{M_L + (b\text{-}1)M_E}$	.47 ± .047	.54 ± .045	.49 ± .046	.36 ± .047	.35 ± .047
	Genetic Components of Variance				
$\frac{M_L - M_E}{b}$ (Total genetic variance)	6.8	10.6	11.9	8.8	12314
Offspring $^1b\ (M_L + M_E)$ (Additive + epistatic)	6.5	9.0	10.3	10.2	10202
Parent-Offspring 2 cov (PO) (Additive + epistatic)	5.2	8.7	8.3	6.7	5901

Heritability estimates for forage yield are comparable with those presented by Wilsie (1951), Davis (1955), and Kehr and Gardner (1960).

The offspring, and parent-offspring data indicate that most of the genetic variation in stem length is caused by additive gene effects. The estimates of additive variance of yield from offspring data were much higher than from parent-offspring data, but the lower estimate indicates that about half of the genetic variance of this character is caused by additive gene effects.

Selection

As the heritability estimates and the estimates of additive genetic variance are high, it is worth while examining the usefulness of the progeny test in this first cycle of selection. In Table 2 the mean of the whole population of progenies, the mean of the top 10% of the progenies, and the mean of the progenies from the top 10% of the parents are compared.

Selection based on parental values has produced progeny populations with as great a mean length of stem as selection based on progeny

TABLE 2. The Effects of Selection, Based on Parents and Progeny
(Population means)

	Length (cm)				Weight (g)
	14 Aug	29 Aug	11 Sep	23 Sep	1 Oct
Unselected progeny	14.0 ± 0.09	22.0 ± 0.11	27.9 ± 0.12	36.0 ± 0.12	451 ± 6.7
Top 10% of progeny	16.8 ± 0.07	25.3 ± 0.25	30.6 ± 0.17	39.3 ± 0.34	556 ± 7.3
Progeny from top 10% of parents	16.5 ± 0.34	24.9 ± 0.38	30.5 ± 0.29	38.3 ± 0.45	480 ± 13.7

performance itself. For this character, the progeny test has been of little use, and more would have been gained by growing and selecting among a much larger parent population than by progeny testing the necessarily much smaller one.

In selecting for weight per plant, selection based on the phenotype of the parent plants has been of very little use, and for advances to be achieved in this character, a progeny test would be necessary. Davis (1955) found consistent differences between polycross yields and performance of synthetics, and poor correlation with parental yield.

Growth Rates

The final length reached by these plants may be separated into two components, the initial height and the growth increment between the first and last measurement. If these were negatively correlated, selection at one time would not be a reliable guide to selection at another. If variations in these components were randomly associated, there would be no correlation between them, and selection based on one would not depress the mean of the other in the selected population. On the other hand if selection were based on a high total of both components, i.e. on final length alone, the two componetns would be negatively correlated in the selected population, even if they were not correlated in the original population, and even though selection had increased the mean of both of them (Wright 1921).

The distribution of initial length and growth increment in the selected and unselected populations of parents is shown in Fig. 1.

In the unselected population of parents, there was a slight negative correlation between initial length and growth increment ($r = -0.22^{*}$). In that half of the population with the greater final length, $r = 0.76^{**}$, and in the lower half $r = -0.40^{**}$. In the unselected population of progenies, $r = -0.02$ in the whole population, -0.19 in the lower half, and -0.70^{**} in the top half.

The relatively small correlation between initial length and growth rate in the unselected population shows that selection at one time is likely to be effective in increasing the mean at another. The negative correlations in the two selected fractions of this population show that

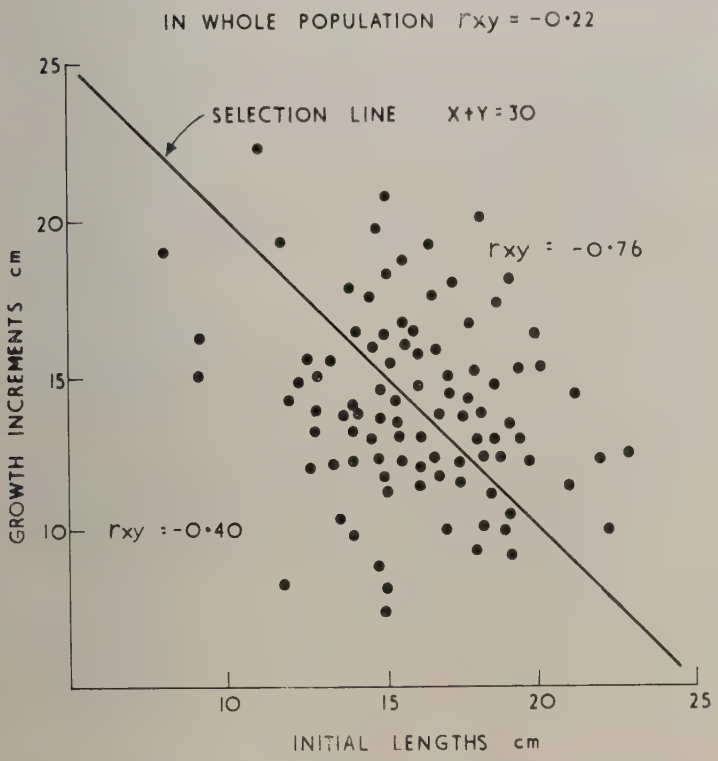

Fig. 1.—Distribution of initial lengths (x) and growth increments (y) in the population of parents.

significant correlation coefficients between characters in selected populations should be accepted with caution as evidence of physiological relationships between them. They may mean little more than that the two characters have been selected together, and if selection were so stringent as to reduce variation in the compound character to zero, the correlation between the components would be 1.0 (Wright 1921).

Conclusions

Considerable genetic variation in winter growth existed in the Hunter River population studied, and phenotypic selection among the plants would be an effective means of increasing the mean length of stems of the population. Selection based on parental phenotypes was not, however, an effective means of increasing the green-matter yield of the population, and for selection for this character to be effective a progeny test would be necessary.

Acknowledgments

The writer wishes to thank Dr B. I. Hayman and Miss E. Stevenson of the Applied Mathematics Laboratory for formulae for heritabilities and for help with computations.

References

Comstock, R. E. 1952: Statistical Genetics in Alfalfa Breeding. *Rep. 13th Alfalfa Imp. Conf., Raleigh, N.C.*, pp. 28–30. (Mimeo.)

Davis, R. L. 1955: An Evaluation of S1 and Polycross Progeny-testing in Alfalfa. *Agron. J. 47:* 572–6.

Kehr, W. R.; Gardener, C. O. 1960: Genetic Variability in Ranger Alfalfa. *Ibid. 52:* 41–4.

Morley, F. H. W.; Daday, H.; Peak, J. W. 1957: Quantitative Inheritance in Lucerne, *Medicago sativa* L. I. Inheritance and Selection for Winter Yield. *Aust. J. agric. Res. 8:* 635–51.

Palmer, T. P. 1959: Winter Growth of Lucerne (*Medicago sativa*) I. Variety performance in New Zealand. *N.Z. J. agric. Res. 2:* 1195–1202.

Wright, S. 1921: Correlation and Causation. I. Method of Path Coefficients. *J. agric. Res. 20:* 557–85.

Wilsie, C. P. 1951: Self Fertility and Forage Yields of Alfalfa Selections and Their Progenies. *Agron. J. 43:* 555–60.

Zaleski, A. 1954: Indentification and Classification of Lucerne Strains. *J. nat. Inst. agric. Bot. 7:* 131–41.

GROWTH OF PASTURE SPECIES IN CONTROLLED ENVIRONMENT

II. GROWTH AT LOW TEMPERATURES

By K. J. Mitchel and Renate Lucanus, Grasslands Division, Department of Scientific and Industrial Research, Palmerston North

(*Received for publication, 2 May 1960*)

Summary

Controlled climate techniques were used to examine the effects of representative winter temperatures on the growth of plants of some important New Zealand pasture species.

Growth of plants in climate cabinets was followed in all combinations of three day temperatures—60°F, 52.5°F, and 45°F, and three night temperatures—45°F, 40°F, and 35°F. The plants received 12 h of light per day at an intensity of approximately 2,700 ft candles and with a spectral composition similar to daylight.

Lower day temperatures gave a much greater relative reduction in growth of individual tillers than did lower night temperatures. In contrast a lowering of day temperatures had a relatively small influence on the rate of tillering of most species but in some cases lower night temperatures resulted in substantially higher rates of tillering.

Introduction

Controlled climate techniques were used to examine the effects on the growth of species and varieties important in New Zealand pastures of temperatures representative of those occurring in various districts during

TABLE 1. Average Daily Maximum Air Temperatures (0°F) and Average Daily Minimum Air Temperatures at Representative Sites in New Zealand During Three Winter Months
(New Zealand Meteorological Service Report)

		June	July	August
Te Paki	Av. Max.	61	59	60
34° 30′S	Av. Min.	48	46	46
Ruakura	Av. Max.	57	56	58
37° 46′S	Av. Min.	38	37	38
Palmerston North	Av. Max.	54	53	55
40° 23′S	Av. Min.	40	39	40
Lincoln	Av. Max.	51	50	52
43° 39′S	Av. Min.	34	32	34
Invercargill	Av. Max.	49	49	56
46° 24′S	Av. Max.	36	34	39

the winter (table 1). The objective of the work was to obtain an estimate of the average rates of vegetative growth under the various temperature conditions, and also an indication of the amount of variation between individual plants in the various species and varieties.

Experimental Methods

These were the same as described in the first paper of this series (Mitchell 1956).

The species examined were: certified New Zealand perennial ryegrass (*Lolium perenne* L.); certified New Zealand short-rotation ryegrass (*Lolium perenne* L. x. *Lolium multiflorum* Lam.); certified New Zealand cocksfoot (*Dactylis glomerata* L.); certified New Zealand browntop (*Agrostis tenuis* Sibth.); Yorkshire fog (*Holcus lanatus*, L.); certified New Zealand white clover (*Trifolium repens* L.); and the Mount Barker variety of subterranean clover (*Trifolium subterraneum* L.) All plants of the five types of pasture grasses and two species of clovers were grown from samples of seed which were considered representative of the material commercially available in the country.

Each of the three day temperatures, 60°F, 52.5°F and 45°F, was combined with each of three night temperatures, 45°F, 40°F and 35°F. The plants received twelve hours of light per day at an intensity of approximately 2,700 ft candles and with a spectral composition close to daylight.

When the young plants had commenced to form tillers, i.e. had grown past the seedling stage, 16 plants of each species or variety were transferred into the climate cabinets and given a period of 10–14 days to adapt to the conditions in that cabinet. A preliminary harvest of 8 of the plants was then taken. The remaining 8 plants were harvested and measured approximately 14 days later to determine the growth during the interval under the respective conditions. With all species and varieties each treatment was repeated several times.

Results

The growth responses of the various species and varieties are summarised in figs. 1 and 2 and tables 2–4. Comments on results are restricted to those features which appeared of most significance to the authors. Although data for rate of growth of the shoot* as a whole are presented, main emphasis is placed on the growth of individual tillers and on rate of appearance of tillers. For reasons outlined previously, these features of the plant's growth are considered the most useful indicators of the responses to be expected from the various species when present in a plant community of the particular structure formed in a grazed pasture (Mitchell and Glenday 1958).

Daily Growth on an Individual Tiller

Where the night temperatures were 35°F instead of 45°F, with day temperatures held at 60°F, there was only a 10–16% less growth on a

* All of the plant which is not root.

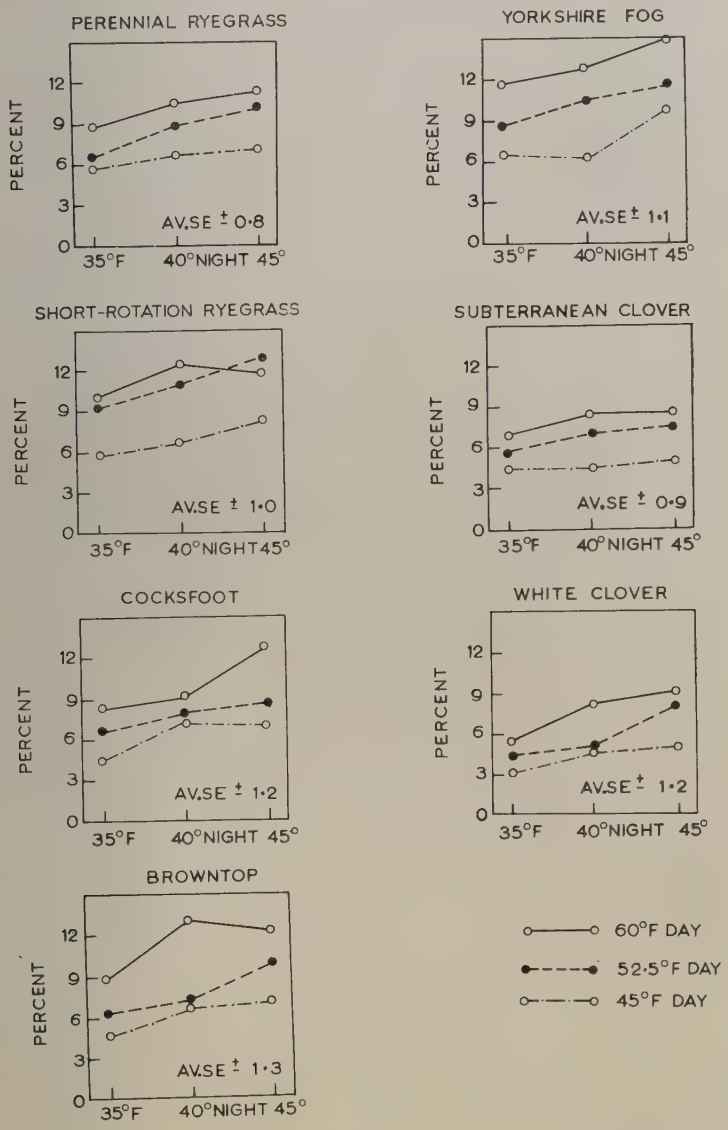

Fig. 1.—Influence of the various levels of temperature on daily growth on a tiller and on rate of tillering.

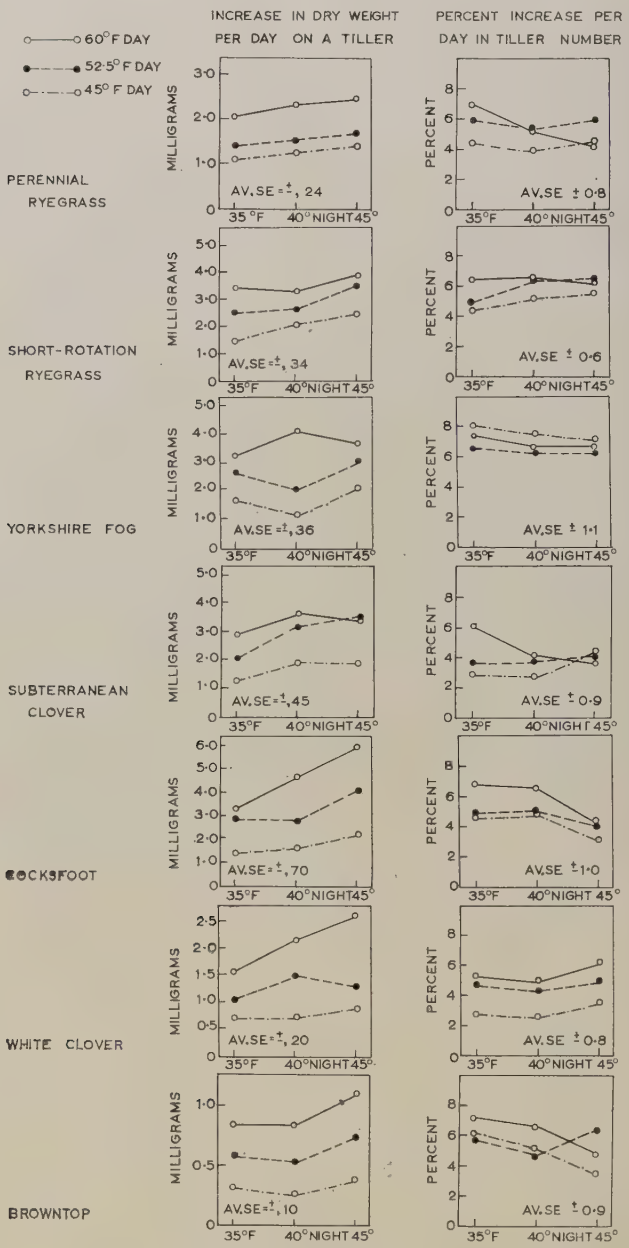

Fig. 2.—Influence of the various levels of temperature on the percentage increase per day in dry weight of the shoot.

TABLE 2. THE RELATIVE CHANGE IN WEIGHT OF TISSUE FORMED PER DAY ON A LEADING TILLER INDUCED BY A LOWERING OF DAY TEMPERATURE OR A LOWERING OF NIGHT TEMPERATURE

Species	Relative Growth				SE
	60°F day 45°F night	60°F day 35°F night	45°F day 45°F night	45°F day 35°F night	
Perennial Ryegrass	100	84	58	45	± 10
Short-rotation Ryegrass	100	88	62	37	± 8
Yorkshire Fog	100	90	59	47	± 9
Subterranean Clover	100	86	58	37	± 13
Cocksfoot	100	57	37	22	± 11
White Clover	100	61	35	27	± 7
Browntop	100	77	37	30	± 9

TABLE 3. THE RELATIVE CHANGE IN RATE OF TILLERING INDUCED BY A LOWERING OF DAY TEMPERATURE OR A LOWERING OF NIGHT TEMPERATURE

Species	Relative Rate of Tillering				SE
	60°F day 45°F night	60°F day 35°F night	45°F day 45°F night	45°F day 35°F night	
Perennial Ryegrass	100	163	107	102	± 17
Short-rotation Ryegrass	100	103	90	71	± 10
Yorkshire Fog	100	110	105	118	± 16
Subterranean Clover	100	167	117	78	± 24
Cocksfoot	100	158	74	109	± 23
White Clover	100	85	60	45	± 13
Browntop	100	150	75	127	± 18

tiller with perennial ryegrass, short rotation ryegrass, Yorkshire fog and the Mount Barker variety of subterranean clover; but there was approximately 40% less growth from cocksfoot and white clover (table 2 and fig. 1). When day temperatures were 45°F instead of 60°F, with night temperatures held at 45°F, the former group gave approximately 40% less growth and the cocksroot and white clover over 60% less growth on a tiller. When both day and night temperatures were lower the differences between the two groups were relatively smaller, but remained present.

The data indicate that browntop is intermediate between the two groups in its response to the lower night temperatures but similar to cocksfoot and white clover in its response to the lower day temperatures.

In addition, the results suggest that there are differences between the two varieties of ryegrass in their response to intermediate levels of day temperature with daily rate of tiller growth in short-rotation ryegrass being less affected by a decline in day temperature from 60°F to 52.5°F

than in perennial ryegrass (fig. 1). This observation is in accord with previous controlled climate observations (Mitchell 1954) and suggests that the major difference between the two types of the ryegrass in response to lower temperature levels occurs at about this range of day temperatures.

There were no significant interactions between day temperatures and night temperatures, i.e. as far as can be judged from the data each exerted its general effect on the growth of tiller independent of the other.

Rate of Tillering

As judged by percentage increase in tiller numbers per day, the responses of these species and varieties to the various levels of temperature were generally very different from those noted for growth on an individual stem (table 3 and fig. 1).

Growing the plants with a night temperature of 35°F instead of 45°F either had little effect on rate of tillering or increased it. The trend for some species to show an increase in rate of tillering at the lower night temperature was most marked when day temperature was held at 60°F. Under these conditions there was a significant increase with perennial ryegrass, cocksfoot ($p = 0.10$), browntop and subterranean clover.

The effect of growing the plants at a day temperature lowered from 60°F to 45°F was, except for white clover, relatively small or absent if the night temperature was held at 45°F; but if night temperature was

TABLE 4. INFLUENCE OF LEVELS OF DAY AND NIGHT TEMPERATURES ON VARIOUS MORPHOLOGICAL CHARACTERS AND ON DRY MATTER CONTENT

Temp. (°F) Day	Night	Perennial Ryegrass	Short-rotation Ryegrass	Yorkshire Fog	Subterranean Clover	Cocksfoot	White Clover	Browntop
(a) Number of days between appearance of successive leaves on a stem								
60	45	10.0	8.3	7.7	6.3	5.9	6.7	7.1
60	35	9.1	8.3	8.3	5.9	7.1	8.3	7.7
45	45	12.5	9.1	9.1	7.7	9.1	12.5	9.1
45	35	11.1	12.5	10.0	11.1	12.5	14.3	11.1
Mean SE		±0.9	±0.9	±1.0	±0.8	±0.9	±0.9	±0.6
(b) Dry matter percentage of the whole shoot								
60	45	15.8	15.3	14.5	16.4	15.5	13.2	19.7
60	35	16.7	15.0	14.9	19.2	16.9	17.2	20.1
45	45	17.5	16.9	16.6	18.4	17.8	17.3	22.6
45	35	19.1	18.2	16.1	22.0	19.3	21.5	22.5
Mean SE		±1.2	±1.1	±0.7	±0.9	±1.4	±1.0	±1.4

TABLE 4 (Continued)

Temp. (°F) Day	Night	Perennial Ryegrass	Short-rotation Ryegrass	Yorkshire Fog	Cocksfoot	Browntop
(c) Dry weight per unit area of leaf blade (mg/cm²)						
60	45	2.86	2.30	2.09	2.33	2.71
60	35	3.16	2.76	2.45	2.57	2.82
45	45	3.32	2.88	2.35	2.62	3.03
45	35	3.89	3.45	2.58	3.09	3.07
Mean SE		±0.24	±0.28	±0.14	±0.25	±0.15
(d) Total leaf elongation—cm per tiller per day						
60	45	2.35	3.53	2.71	3.92	1.55
60	35	1.93	2.76	2.00	2.20	1.11
45	45	1.30	1.86	1.42	1.50	.57
45	35	.96	1.18	1.07	.89	.46
Mean SE		±0.15	±0.15	±0.19	±0.38	±0.13
(e) Final average length of leaf blades (cm)						
60	45	15.5	20.2	14.4	17.5	8.6
60	35	13.2	16.3	12.2	12.3	6.3
45	45	11.9	14.9	10.2	9.4	4.1
45	35	9.7	10.8	9.2	6.7	4.0
Mean SE		±1.4	±1.6	±1.5	±2.4	±0.9
(f) Final average width of leaf blades (mm)						
60	45	3.69	4.85	6.56	6.48	2.91
60	35	3.19	4.41	6.54	5.82	2.77
45	45	3.31	4.45	6.45	5.66	2.36
45	35	2.87	3.58	5.86	4.77	2.30
Mean SE		±0.20	±0.28	±0.47	±0.42	±0.23

35°F, reduction in day temperature from 60°F to 45°F generally substantially reduced rate of tillering.

General Growth of the Shoot

It was apparent that the rate of growth of the shoot as a whole (fig. 2) was determined mainly by the influence of various levels of day and night temperatures on the amounts of tissue formed per day on individual tillers rather than by their effect on the rate of tiller production (c.f. fig. 1).

General Morphological Characters and Dry Matter Content

Table 4 summarises the data indicating the influence at various levels of temperatures on other features of growth of the plants. Both lower day and lower night temperatures induced trends for an increase in dry-matter percentage of the shoot, an increase in dry weight per unit area of leaf blade, and a reduction in final average width and length of the leaf blades. Such trends are in accord with previous findings with these and other species of pasture grasses (Peterson and Loomis 1949; Hiesey 1953; Langer 1954; Mitchell 1956).

The interval between the appearance of successive leaves on stems of some species, e.g. cocksfoot and white clover, was markedly longer at lower day or night temperatures, but in other species, e.g. perennial ryegrass, the effect of temperature reductions was comparatively small.

For the grasses the rate of elongation of leaves on a stem was a relatively good index of overall rate of tissue formation on a tiller—under the temperature and light conditions used in this work. Increases in dry weight per unit area of leaf blade resulting from lower temperatures were balanced by decreases in width of the leaf blades.

Discussion

The finding that within the range of temperatures used it is the level of day temperature rather than the night temperature which has most influence on the daily growth of individual tillers, is of importance for understanding the effects of various sets of weather conditions on winter productivity of pastures. It is considered that rates of growth of individual tillers will be a reasonably good index of the relative amounts of daily growth to be expected from an established pasture at the various levels of day and of night temperature.

The lack, for most of the species examined, of any restriction on the rate of appearance of new tillers caused by cooler temperatures and also the apparent stimulation of tillering in some species by cool nights provided the days are reasonably warm, suggests that the cool period of the year may be regarded as a time when a considerable build-up in tiller density of a pasture can occur. Field evidence in accord with such deductions has recently been presented by Langer (1959). He found, in England, that a substantial build-up in tiller numbers can occur in pastures during late autumn and early winter.

Earlier Peterson and Loomis (1949) had commented on thickening of bluegrass (*Poa pratenis*) swards during autumn. As they had found tillering of this species was stimulated by a reduction in photoperiod to 11 hours, c.f. 15 and 19 hours, rather than lower temperatures, they drew particular attention to the importance of the shortening of the days in inducing the observed thickening of bluegrass swards. However, the average minimum temperature in their cool treatment was 55.8°F. The results from the present work indicate that tillering and consequent increase in density of a pasture will also be stimulated by the onset of cool nights where temperature falls below 40°F such as occurs frequently during autumn.

As Jacques and Edmond (1952) have found that in New Zealand, the major flush of root development occurs before the commencement of the period of rapid growth of herbage during spring and early summer, the evidence overall suggests that the course of development of a pasture during the cool period of the year can be of particular importance in determining its potential for high production during the subsequent spring and early summer. If individual plants are given reasonable opportunity to develop by not being grazed too hard or too laxly, both the relatively high rates of tillering and the flush of root growth which can occur could lead to the establishment by early spring of a sward which has a high density of young tillers and a vigorous root system. Given adequate mineral nutrition, such a pasture should produce high yields of herbage during the spring and early summer, without the finer details of management procedure being of critical importance.

Acknowledgment

Thanks are due to Miss V. Fleming, Applied Mathematics Laboratory, for statistical examination of the results and Prof. R. H. M. Langer for discussion of results.

References

Hiesey, W. M. 1953: Growth and Development of Species and Hybrids of Poa under Controlled Temperatures. *Amer. J. Bot. 40:* 205–21.

Jacques, W. A.; Edmond, D. B. 1952: Root Development in Some Common New Zealand Pasture Plants. V. The Effect of Defoliation and Root Pruning on Cocksfoot (*Dactylis glomerata*) and Perennial Ryegrass (*Lolium perenne*). *N.Z. J. Sci. Tech. A34:* 231–48.

Langer, R. H. M. 1954: A Study of Leaf Growth in Timothy (*Phleum pratense*). *J. Brit. grassl. Soc. 9:* 275–84.

—— ——1959: Growth Pattern of Grasses in the Sward. *Proc. N.Z. Inst. agric. Sci.* 1959, pp. 53–9.

Mitchell, K. J. 1956: Growth of Pasture Species under Controlled Environment. I. Growth at Various Levels of Constant Temperature. *N.Z. J. Sci. Tech. A38:* 203–16.

Mitchell, K. J.; Glenday, A. C. 1958: The Tiller Population of Pastures. *N.Z. J. agric. Res. 1:* 305–18.

Peterson, M. L.; Loomis, E. W. 1949: Effects of Photoperiod and Temperature on Growth and Flowering of Kentucky Bluegrass. *Plant Physiol. 24:* 31–43.

RESPONSE OF SOME NEW ZEALAND PASTURE SPECIES TO VERNALISATION

By S. O. Fejer, Grasslands Division, Department of Scientific and Industrial Research, Palmerston North

(*Received for publication, 19 May 1960*)

Summary

The effects on flowering of vernalisation of germinated seeds have been investigated in some pasture species, with particular reference to varietal differences.

In all ryegrass varieties tested, 90 days' treatment at 3°c induced full flowering, and no flowering occurred in the unvernalised controls. At intermediate durations of cold treatment, there were differences in response both between and within varieties, indicating that vernalisation could be a useful adjunct to pasture plant breeding.

In red clover, cowgrass required no vernalisation, while Montgomery red clover flowered fully following 45 days' treatment. The usefulness of cold treatment of germinated seeds, or growing plants, for varietal separation is discussed.

In timothy, there were varietal differences in time of flowering under the competitive conditions of the trial, but vernalisation had no effect.

Introduction

Vernalisation (exposure of germinating seeds to low temperature) has a number of applications in pasture plant breeding. For example, earlier flowering can speed up breeding programmes or distribute work more evenly throughout the year (Wit *et al.* 1955). It can also differentiate between morphologically similar but physiologically different populations, as reported recently by Cooper (1957). This may help in varietal certification and also in plant selection within a variety.

Because of the importance of these two applications, the effects of vernalisation on varieties of some of the pasture plants of economic importance in New Zealand were examined.

Material

The following species and varieties of pasture plants were used:

Ryegrass (*Lolium* spp.)

N.Z. pedigree perennial ryegrass (Ba), N.Z. pedigree short-rotation ryegrass (Cg), N.Z. pedigree Italian ryegrass (Bb), F2 derivatives of N.Z. perennial ryegrass × N.Z. short-rotation ryegrass crosses (Ba × Cg), and two selections within perennial ryegrass termed "Pasture" and "Hay".

Red Clover (*Trifolium pratense*)

N.Z. pedigree cowgrass (early flowering), N.Z. pedigree Montgomery red clover (late flowering) and four commercial samples of each of these two varieties.

Timothy (*Phleum pratense*)

Two N.Z. pedigree selections listed as Grasslands 1952 and 1955, Welsh S 48, an uncertified N.Z. sample, an American commercial variety, and German Odenwalder.

EXPERIMENTAL METHODS

The effects of different periods of vernalisation at 3°C were investigated for the 6 ryegrasses and the 10 red clover varieties in 1955, and for the 6 timothy varieties in 1957.

In each year, the first of the seeds to be treated were germinated during the first week in July, on moist filter paper in petri dishes at 20°C for 24 hours and were then transferred to a refrigerator at 3 °C. Samples of seed of each ryegrass and red clover variety were germinated and cold treatments begun at two-weekly intervals in 1955, in 1957 the timothy samples were germinated at three-weekly intervals. Occasional uncovering of the petri dishes provided for aeration and the filter papers were kept moist. All seedlings were planted out at the same time, during the first week of October 1955 for the ryegrasses and red clovers, and during the first week of September 1957 for the timothy varieties, along with control seedlings germinated 24 hours earlier. The seedlings were planted into shallow boxes at 3 in. intervals, in a split-plot design, vernalisation periods in main plots, varieties in sub-plots, in 3 replications of 4 plants per plot in 1955 and in 5 replications of 2 plants per plot in 1957. The seed boxes were placed outside under naturally increasing daylengths, and the plants were examined twice a week for inflorescence emergence.

The method of cold treatment deviated from the original concept of vernalisation, as different extended cold periods were used, and no attempt was made to restrict the growth of the seedlings. Essentially the same method was applied to ryegrasses by Cooper (1957) and by Wit *et al.* (1955), and to other plants by other workers. All use the term "vernalisation" to describe their methods, and this term will be used in the same sense in the present paper. It should be noted, however, that with this technique some growth must occur during the longer periods of vernalisation and that this growth can vary with different varieties. If the growth of the coleoptile can shorten the required period of cold treatment, as observed in wheat by Chinoy (1956), this interaction could interfere with the comparisons between responses of different varieties, a limitation that has to be kept in mind.

The close spacing of the plants in the boxes may have retarded inflorescence emergence as can occur in ryegrasses (Fejer 1959). This spacing made conditions closer to those in a pasture than those used by other workers, but the results are not strictly comparable with theirs, e.g., with those obtained with the greenhouse technique of Wit *et al.*

RESULTS AND DISCUSSION

Ryegrasses

Results. The percentages of plants which had flowered in the different varieties after vernalisation are shown in Table 1.

TABLE 1. Percentage of Flowering Ryegrass Plants after Vernalisation at 3°C. December 1955—January 1956

Cold Treatment (days)	Perennial Ryegrass Selections		Ryegrass Varieties				Mean
	"Pasture"	"Hay"	Ba	Ba x Cg	Cg	Bb	
90	100	100	100	100	100	—	100
75	92	100	—	—	100	91	96
60	83	100	75	75	50*	92	79
45	67	70	33	67	73	67	63
30	33	55	17	18	67	40	38
15	0	8	0	0	25	0	5
0 (Control)	0	0	0	0	0	0	0

Analysis of variance (without treatments at 90 and 0 days), transformed data

Source of Variation	d.f.	Mean Squares
Varieties	5	370.5 ($p = 0.03$)
Treatments	4	4,391.5 ($p = 0.001$)
Variety x Treatment	18	99.2 ($p = 0.10$)
Theoretical variance	∞	68.4

* One replication only

Under the conditions of the trial, 90 days vernalisation produced full flowering in all varieties and selections by 16 January. It should be noted that the plants in the control treatments were germinated in the first week of October, 15 weeks before the final examination; the lack of flowering in plants subjected to this treatment, therefore, cannot be taken as absolute proof that all the ryegrasses tested must have at least some cold treatment.

The analysis of variance in Table 1 shows that there were significant differences *between* varieties in their vernalisation requirements. This requirement was high in pedigree perennial ryegrass (Ba), lower in Italian (Bb) and even lower in their hybrid derivative, short-rotation ryegrass (Cg). The hybrid between perennial and short-rotation ryegrasses (Ba × Cg) behaved like perennial ryegrass in the shorter vernalisation treatments. *Within* perennial ryegrass, the "Hay" selection needed only 60 days vernalisation for full flowering, in contrast to the "Pasture" selection and the pedigree perennial variety, both of which required 90 days vernalisation.

Discussion. Wit *et al.* (*op. cit.*) state that raising two generations of perennial ryegrass in a year is not practicable, as the cold requirement for full flowering is more than two months. The present results confirm this conclusion. Even New Zealand Italian ryegrass was no better in this respect, a result which differs from that of these workers.

The differentiation between and within varieties closely parallels the results of Cooper (1957), who found that in *Lolium* the degree of perenniality is related to vernalisation requirements. Between varieties, the

shorter-lived Italian ryegrass showed a lower degree of cold requirement than perennial. Within perennial ryegrass, the "Pasture" and "Hay" material was from open pollinations in the second cycle of selection for high and low herbage yield resulting in such types (Fejer, 1955). The "Hay" type, which is shorter-living (Fejer, in preparation), needed a shorter period of vernalisation for full flowering than did the "Pasture" type.

The "Hay" type is earlier flowering than the "Pasture" type, but the relationship between earliness and perenniality is different between varieties, since Italian ryegrass is later flowering than perennial.

There was therefore potential variability for vernalisation requirement in the original population. As there were correlated responses to selection for herbage yield between this character and earliness and perenniality, vernalisation requirement could be a useful tool for the plant breeder. Similarly, Cooper (1957) has made a preliminary report of the correlated response to within-strain selection for earliness of leaf number before heading in Wimmera ryegrass.

Red Clovers

Results. The effects of the cold treatment on percentage of flowering plants in the red clover varieties are shown in Table 2.

TABLE 2. PERCENTAGE OF FLOWERING PLANTS IN RED CLOVER AFTER VERNALISATION AT 3°C. JANUARY—MARCH 1956

Cold Treatment (days)	Cowgrass Samples					Montgomery Samples					
	Pedigree	1*	2	3	4	Pedigree	1*	2	3	4	Transformed means
45	100	100	100	100	100	100	100	100	100	100	90 ± 7
30	100	100	100	100	100	89	86	67	100	100	75 ± 7
15	100	88	100	100	100	87	100	100	60	90	74 ± 7
0 (Control)	100	100	100	80	100	50	82	91	91	86	65 ± 7

Analysis of variance (Montgomery clover only, transformed data, and omitting 45-day treatment).

Source of Variation	d.f.	Mean Squares
Samples	4	97.0 ns
Treatments	2	160.3 ns
Samples x Treatments	8	265.4 (p = 0.01)
Theoretical variance	∞	82.1

* 1, 2, 3, and 4 = Commercial samples

The results for treatments of longer than 45 days are not shown in Table 2 as many cowgrass plants died when planted, but with these longer treatments all surviving plants flowered. The earlier flowering cowgrasses showed virtually full flowering in the controls and were not included in the statistical analysis.

Only half of the plants flowered in the unvernalised pedigree Montgomery variety. Under the conditions of the trial this variety needed more than 30 days vernalisation for full flowering. As shown in the analysis of variance in Table 2, there was a significant trend to less flowering with shorter cold treatments in all samples.

Discussion. Differences in vernalisation requirements have not been reported previously for the red clovers, but in subterranean clover (*Trifolium subterraneum*) Aitken (1955) found that the early flowering Dwalganup variety has a lower vernalisation requirement than the later Tallarook. This relationship between earliness and vernalisation requirement is similar to that in the two selections *within* perennial ryegrass, but the reverse of the relationship *between* pedigree perennial and Italian ryegrasses. Further, the relationship between perenniality and vernalisation requirement, found in the ryegrasses, is indicated in the red clover varieties, where cowgrass (with no vernalisation requirement) is generally regarded as the shorter-lived variety (Corkill, 1949).

Aitken also found that the vernalisation requirement of subterranean clover can be replaced by long photoperiods. The same result can be deduced from the work of Gorman (1955) with red clover varieties. He found that, without any cold treatment, both early and late varieties flowered fully in a 14-hour photoperiod, while with a 12-hour photoperiod all 16 plants of cowgrass flowered but only two of the 16 plants of Welsh S 123 had flowered six months after planting.

Gorman's work was an attempt to obtain an early and clear-cut differentiation between the two varieties, cowgrass and Montgomery red clover. He also tried chilling 45-day-old seedlings for six days at 3°C but found this of no help. This can perhaps be explained by the present results. Any vernalisation would only lessen the difference between the two varieties in percentage of plants flowering while at the same time shortening the vegetative cycle of both.

The flowering date details are not shown in the tables, but it is noteworthy that the mean flowering date of pedigree Montgomery red clover was nine days later than that of the four commercial samples, with a range overlapping that of the pedigree cowgrass.

Timothy

Results. The trial was terminated at the end of January 1958, and the percentages of plants flowering up to that date are shown in Table 3.

The results show that there were no differences in response to vernalisation, but the variety differences vere highly significant. The less persistent and less leafy New Zealand uncertified and the American varieties contained more heading plants than the Welsh pasture-hay variety S.48, while the New Zealand pasture-hay varieties showed even less heading. The higher number of plants flowering in the 1955 Grasslands selection can be explained by a conscious narrowing of the flowering range in this selection.

Discussion. Langer (1955) reported lack of response to vernalisation in the Welsh S.48 timothy. In New Zealand, in spaced spring plantings

TABLE 3. PERCENTAGE OF FLOWERING PLANTS IN TIMOTHY STRAINS AFTER VERNALISATION AT 3°C. JANUARY 1958

Cold Treatment (days)	Grasslands 1952	Grasslands 1955	New Zealand Uncertified	U.S.A.*	Welsh S.48	German Odenwalder
63	10	30	90	100	50	90
42	10	20	60	100	30	70
21	30	30	90	100	30	90
0 (Control)	0	10	100	100	40	100

Analysis of Variance (transformed data)

Source of Variation	d.f.	Mean Squares and F Tests
Varieties	4	2,583 (p = 0.001)
Treatments	3	147 ns
Variety x Treatment	12	139 (p = 0.07)
Theoretical variance	∞	.82

* U.S.A. data excluded from analysis

in the field of the 1952 and 1955 Grasslands selections significant differences were found between these two selections and also between them and other varieties, in the number of plants flowering in the sowing year, but not in the following year. It is clear that the differences observed in the field were not due to vernalisation responses.

The present results show differences in earliness between varieties but had the trial continued through February, more flowering would have been expected in most varieties as the photoperiodic threshold had already been passed.

In general the results are in agreement with those of Cooper (1957) who used some of the same varieties. A major difference however is in the position of the Odenwalder timothy variety. While Cooper reported it to be slower heading than the Welsh S.48 variety, and classified it with some very late Scandinavian material, the present trial ranked Odenwalder with the rapid heading uncertified New Zealand material, both being nearly as early as the variety from America. If it is assumed that the Odenwalder variety is uniform in earliness from harvest to harvest and from different seed sources, the explanation of these differences, as well as those between the New Zealand Grasslands varieties in the sowing and second years, could lie in factors other than vernalisation or photoperiod. Similar conclusions have been reached about sowing year and first harvest year differences by Cocks (1958). One possible factor, the inhibition of heading by high temperatures, has been reported recently by Cooper (1958).

ACKNOWLEDGMENTS

Grateful acknowledgment is given to colleagues at the Grasslands Division, to Mr A. C. Glenday, Applied Mathematics Laboratory, for help

with layouts and analysis, and Dr R. G. Thomas, Plant Physiology Unit, for helpful discussions, and to Mrs M. Johnston, Seed Testing Station, Department of Agriculture, Palmerston North, for the commercial samples of red clover varieties.

References

Aitken, Yvonne 1955: Flower Initiation in Pasture Legumes. *Aust. J. agric. Res. 6:* 212.

Chinoy, J. J. 1956: Determination of Photoperiodic and Vernalisation Quanta for the Vegetative Period of Wheat. *Physiol. Plant. 9:* 1.

Cocks, Betty 1958: The Influence of Strain and Date of Sowing on Head Production and Seed Yield in S.48 Timothy. *J. Brit. grassl. Soc. 13:* 92.

Cooper, J. P. 1957: Development Analysis of Populations in the Cereals and Herbage Grasses. II. Response to Low Temperature Vernalisation. *J. agric. Sci. 49:* 361.

———1958: The Effect of Temperature and Photoperiod on Inflorescence Development in Strains of Timothy (*Phleum* spp.). *J. Brit. grassl. Soc. 13:* 81.

Corkill, L. 1949: Pasture Improvement in New Zealand. *Emp. J. exp. Agric. 17:* 157.

Fejer, S. O. 1955: Genotype-Environment Interactions in *Lolium perenne. Nature, Lond. 175:* 944.

———1959: Intraspecific Competition as a Factor in Ryegrass Breeding. *N.Z. J. agric. Res. 2:* 107.

Gorman, L. W. 1955: Effect of Photoperiod on Varieties of Red Clover. (*Trifolium pratense* L.) *N.Z. J. Sci. Tech. A37:* 40.

Langer, R. H. M. 1955: Ear Formation in Timothy Grass (*Phleum pratense*) following Vernalisation and Short-day Treatments. *Nature, Lond. 176:* 263.

Wit, F.; Margadant, W. D.; Dilz, K. 1955: Jarowisatie bij engels en Italiaans raaigras. *Landbouwk. Tijdschr. 67:* 137.

CHANGES IN THE PORE-SPACE OF A PASTURE TOPSOIL UNDER ANIMAL TREADING

By M. W. Gradwell, Soil Bureau, Department of Scientific and Industrial Research, Wellington

(*Received for publication, 12 May 1960*)

Summary

Changes in the Pore-space of the top 1½ in. of Manawatu silt loam under pasture were followed in three successive winters. On each sampling date twelve hand-carved cores were taken from randomly disposed sites in a paddock grazed by sheep. The content of pores drained at a suction of 50 cm of water was determined, and also the volume of water drawn from the saturated cores by this suction. Soil structure, as measured by these coefficients, fluctuated considerably within the space of a few months, the least drainage being found after periods of heavy grazing in wet weather. In one case the content of pores drained at 50 cm of suction fell from 10% to 3% of the soil volume as a result of grazing during the winter period. The volume of pores drained by this suction rose again following the onset of drier weather, but only after a time-lag of 1–2 months in the cases studied. No relationship was found between these changes in pores drained by 50 cm of suction and the fluctuations of total pore-volume which took place in the same paddocks.

Soil cores containing the greatest weight of roots had the highest measured volumes of pores; on some occasions they also showed a tendency to drain more than cores containing fewer roots.

Introduction

Many investigations have been made into the effect of pasture leys on the structure of cropping soils. However, as Alderfer and Robinson (1947) state, "The structure of pasture soils has not as yet received much direct attention from investigators". Most of the work which has been done on the effect of grazing on soil structure has taken the form of comparisons between heavily grazed pastures and grassland subjected to light or zero grazing.

On a gleyed silt loam in Latvia, Kudelin (1952) compared the 0–5 cm depth of pasture and hay treatment. Soil porosity and air-space were considerably lower under pasture. Air-space increased down the profile, especially under pasture. McIntyre (1957) and Tanner and Mamaril (1959) compared trodden and untrodden grassland in Wisconsin. They found lower porosities and lower volumes of air pores in the 0–3 in. depth of the trodden soils. O'Connor (1956) found that, when freshly mown grassland was trodden at intervals through a season by dairy cows, the surface 1.5 in. of soil was appreciably compacted. On unmown grassland the compaction was less. Irrigation before treading did not increase compaction. This was on a silty clay loam containing 9% of organic matter.

Alderfer and Robinson (1947) compared different intensities of grazing on a clay loam on limestone. On heavily grazed sites the air capacity (air-space volume at field capacity) of the 0–1 in. depth was lower than on lightly or moderately grazed sites. Recently, Edmond

(1958a) has also found lowered total porosities in the upper 6 cm of Manawatu silt loam with increased intensities of treading by sheep.

A little work has been done on seasonal changes in the porosity of pasture topsoils. Dreibelbis and Post (1943) found that porosities in the 0–3 in. depth of a silt loam were lower in the late summer than in the winter. Heinonen and Pukkala (1954) worked on the surface 5 cm of a second year ley and a fifth year pasture on a clay loam in Finland. They also found minimum porosities in the late summer, when the soil was driest.

It has been pointed out that production on the high-fertility pastures of New Zealand may be limited by puddling of the surface soil in wet weather (Taylor, 1954). Accordingly it seemed that more information should be accumulated on the range and rate of change of soil structure to be found under winter and spring conditions in a single heavily-stocked pasture.

Grazing Conditions and Sampling Plan

Undisturbed samples of the surface soil were taken from the farmlet area at Grasslands Division, Department of Scientific and Industrial Research, Palmerston North, during winter and spring of the three years 1957–59. The soil type here is Manawatu silt loam, a deep, freely-draining, recent soil containing about 4% of organic carbon in the topsoil. The natural fertility of this soil is high to very high (Soil Bureau, 1954).

The paddocks sampled were almost level and were sown to pastures of ryegrass and white clover and grazed by sheep. A different paddock was sampled in each year of the work. The paddocks employed in 1957 and 1958 were set-stocked throughout the period of the experiment at a level generally determined by the feed available.

TABLE 1. Monthly Rainfall Totals During Sampling Periods (in.)

Year	June	July	August	Sept.	October	Nov.
1957	3.80	2.98	1.18	2.80	5.01	—
1958	2.60	2.91	5.84	0.50	2.34	1.31
1959	—	1.81	1.22	—	—	—
Normal rainfalls adjusted to period 1921–50	3.9	3.1	3.5	2.9	3.8	3.3

The first sampling in 1957 was made on 18 June. About ten days earlier the paddock had carried a large mob of sheep for 36 hours in wet weather. Set-stocking had begun four days before the sampling. The second sampling was made on 12 August. At this time the pasture appeared damaged by heavy grazing in wet weather three weeks earlier. Patches of bare soil several inches in diameter were visible and stock had been temporarily removed to permit the pasture to recover. Soon after the sampling set-stocking was resumed. Two further samplings were made on 30 September and 21 October.

The paddock used in 1958 was first sampled on 16 June, at the end of a period of spelling which had continued since it was grazed by a

mob, under dry conditions, at the end of April. Grazing was lenient for the next five weeks, with sheep admitted for short periods only in fairly dry weather. Set-stocking began on 24 July and was intensified in August, as the soil grew wetter. It was maintained at about the same intensity through September, by which time the soil was drying. Samples were taken on 28 July, 11 August, 1 September, 6 October, 3 November and 8 December. No obvious deterioration of the pasture was noted in the winter of this year.

The paddock employed in 1959 was grazed by a mob when the soil was wet and sampled immediately afterwards, on 27 July. Thereafter no grazing occurred during the sampling period. Three more samplings were made at intervals of a fortnight.

Monthly totals of rainfall at Grasslands Division during the sampling periods are set out in Table 1, together with normal totals for the corresponding months, obtained from the records of thirty years.

METHODS

The index of soil structure employed in this work was the volume of pores drained at a suction of 50 cm of water ("free-draining pores".) At each sampling twelve cores about 4½ in. in diameter and 1½ in. deep were carved by hand from the surface soil at randomly located sites in the experimental paddock. Live worms in the cores were extracted with tweezers as soon as possible. The samples were brought to the Laboratory in sealed cans and the cores for structure testing cut from their centres by driving into them, by means of a screw press, brass cutting cylinders 3 in. in diameter and 0.9 in. long. These cores corresponded to the 0.3–1.2 in. depth of the soil in the field. The driving was carried out in stages of about 0.2 in. and at the conclusion of each stage the soil was carefully cut for 0.2 in. ahead of the cutting edge into a cylindrical surface along which the edge would travel, all roots being severed with a razor blade. As in previous work (Gradwell, 1958) any particles of soil torn from the cylindrical surfaces of the core during this process were replaced by remoulded material. The soil at either end of the cylinder was cut to a plane surface as tested by a straight-edge. The cores were wetted from the bottom up and soaked overnight in dishes of water. They were then quickly dabbed dry on the outside with a cloth, weighed, and pressed onto a filter paper in a Buchner funnel. Through the paper they made contact with a continuous column of water leading to an open surface 50 cm below the soil sample. The top of each Buchner funnel was covered with a plastic sheet and a square of insulating wallboard to minimise evaporation losses from the samples. After draining for 24 hours the cores were removed and weighed again. Any pieces of soil which had been torn from their plane surfaces during cutting were now replaced by remoulded material. The weights of solids and water in the cores after drainage were next determined by drying them in an oven at 105 110°c and reweighing. In each year's work a mean specific gravity for the soil in the paddock was determined and this figure was applied to every core to compute its volume of solids. The volume of solids and water were added and the remaining part of the volume of the cores was recorded as pores drained at 50 cm of suction. The loss of weight during drainage was recorded as water drained from

the saturated soil by 50 cm of suction. Both of these quantities were expressed as percentages of the volume of the whole core. The percentage of the core volume not occupied by solids was recorded as porosity. At some samplings the weight of the core at its field moisture was recorded after carving and before saturating it. The difference between this and its weight after drainage was used to compute the volume of air-space in the field as compared with the volume of pores drained at 50 cm of suction.

The reproducibility of the core-cutting technique was tested by making up blocks of uniform soil in a consolidation press. Slurried soil was compressed under a pressure of 0.5 ton per sq. ft with free drainage allowed from both ends of the sample. From the resulting large core three cores were carved by the procedure described above and their porosities and volumes of air-space determined. The mean porosity was 54.7%, standard deviation, 0.23%; mean air-space, 2.7% standard deviation, 0.21%. Some small bubbles of air were visible in the cores. This experiment was repeated twice, using pressures of 1 and 2 tons per sq. ft. In these tests the standard deviations of porosity and air-space were even lower than those from the first test. It was concluded that on uniform soils the core-cutting techniques employed gave extremely reproducible results. With field samples varying contents of roots complicate the cutting and probably lead to some artificial widening of the range of variation of replicates. Nevertheless, the writer considers that the much greater variations found in the field are mainly due to natural soil variability in the paddocks sampled.

Manawatu silt loam does not display marked signs of swelling when wetted. The influence of wetting and drying on porosity was tested in January, when the soil was drier than at any time during the samplings of the investigation. Several patches of a paddock were irrigated and six cores taken from the irrigated areas were compared with the dry areas of the paddock. The mean porosity in the wetted areas exceeded that in the dry by 1.4% of porosity, a barely significant difference. There was no significant difference in pores drained at 50 cm of suction nor in the volume of water removed from saturated cores by this suction. It was concluded that such variations of soil moisture as occurred between sampling dates would not have any appreciable effect on the volume of pores drained in the laboratory.

It was thought that there might be some danger of spurious differences arising between sampling dates because of changes in the properties of the soil samples during storage. To minimize this, samples were always tested according to a set timetable, three cores being carved and soaked on each of the four days following a sampling. A comparison was made of the results derived from cores carved on the first and second days after sampling and those carved on the third and fourth days. Over all sampling dates the cores stored longer had a content of free-draining pores or of water drained about 0.5% lower than did those tested first. However, the relationship was not sufficiently consistent to show any definite change of soil properties with storage.

Determinations of the quantity of roots present in the test cores were made on some occasions. The oven-dried cores were soaked in water, then broken up by hand and gently rubbed through an 18-mesh

TABLE 2. MEAN STRUCTURAL PROPERTIES OF SOIL ON SAMPLING DATES IN 1957
(0.3–1.2 in. depth)

Date	Porosity (%)	(S.D.)	Volume of pores Drained at 50 cm of suction (%)	(S.D.)	Water drained by 50 cm of suction (%)	(S.D.)	Estimated vol. worm-holes (%)	(S.D.)	Prevous Weather and Grazing Conditions
18 June	57.1	(1.9)	5.4	(1.3)	6.1	(0.8)	1.9	(0.6)	One brief heavy stocking in wet weather.
12 Aug.	55.7	(1.5)	3.0	(0.9)	4.3	(0.6)	1.6	(0.5)	Heavily grazed in wet weather then a spell.
30 Sept.	58.6	(2.3)	4.9	(0.9)	4.9	(0.6)	1.3	(0.3)	Drier weather; set-stocked.
21 Oct.	57.7	(2.3)	3.1	(1.6)	3.9	(1.3)	1.5	(0.6)	Exceptionally wet weather; set-stocked.

TABLE 3. Mean Structural Properties of Soil on Sampling Dates in 1958 (0.3–1.2 in. depth)

Date	Porosity (%)	(S.D.)	Volume of Pores Drained at 50 cm of Suction (%)	(S.D.)	Volume of Water Drained by 50 cm of Suction (%)	(S.D.)	Soil Moisture at 50 cm of Suction (Dry weight basis %)	Appearance of Soil	Weight of roots in 100 ml of soil (g)	Previous Weather and Grazing Conditions
16 June	61.8	(2.7)	9.5	(3.1)	9.6	(2.9)	54.6	Granular, friable, rather dry	—	Spelled for several weeks
28 July	62.1	(2.3)	6.3	(1.5)	7.1	(1.5)	58.6	Less granular and wetter	0.68	Light grazing under dry conditions
11 Aug.	62.3	(2.4)	4.6	(1.1)	5.6	(0.9)	61.2	Compact, massive and wet	0.77	Set stocking
1 Sept.	62.7	(2.8)	2.9	(1.2)	4.3	(1.1)	64.2	Massive, compact, wet, signs of gleying	0.81	Heavier stocking and wet conditions
6 Oct.	56.3	(1.7)	2.9	(2.7)	5.3	(2.5)	48.6	Massive, dense and hard, dry	0.79	Continued heavy stocking; drier soil
3 Nov.	57.3	(1.8)	4.7	(1.9)	4.6	(0.9)	48.9	Massive, dense, wetter	—	Soil drying out
8 Dec.	55.4	(1.9)	7.2	(1.8)	6.3	(1.6)	43.0	Divided by cracks into small fitted aggregates	—	Soil dry

(085-mm aperture) sieve until all soil was removed from the roots remaining on the sieve, which were oven-dried and weighed. The finer fragments of roots lost through the sieve appeared to represent a minor portion of total root weight.

To estimate the volume of wormholes advantage was taken of the fact that at the depth from which the test cores came these ran mostly in a vertical direction. Oven-dried cores were cut in half in a horizontal plane with a hacksaw. The wormholes intersecting the plane of the cut were counted and their diameters measured. By assuming that they ran the full depth of the core their aggregate volume was computed. This was increased by a small factor to allow for the shrinkage of the core on drying.

RESULTS

The mean results for each sampling date in 1957 are set out in table 2. In this year August was an abnormally dry month and October abnormally wet; the other months of the sampling period had normal rainfalls.

The porosity decrease between 18 June and 12 August was barely significant statistically but the increase to 30 September was highly significant. The changes in volume of pores drained at 50 cm of suction were highly significant in each interval between successive samplings. The decrease in volume of water drained between 18 June and 12 August was highly significant and the changes in the remaining two intervals reached significance.

The mean results for each sampling date in 1958 are set out in table 3. In this year August was a very wet month and September abnormally dry; otherwise rainfall conditions during the sampling period did not deviate greatly from average. Soil porosity did not change up to 1 September but between this date and 6 October a decrease was recorded which was highly significant. The next significant difference was recorded between 3 November and 8 December, when a further decrease in porosity occurred. The volume of free-draining pores varied with time in a manner quite different from that of total porosity. In each of the first three intervals between samplings a highly significant decrease was recorded until the minimum value was attained on 1 September. The figure for 3 November represented a significant rise above this minimum and the larger increase from 3 November to 8 December was highly significant. The volume of water drained varied similarly to the content of free-draining pores. The decreases in the first two intervals were significant and in the third, highly significant. The first appreciable increase above the minimum volume was recorded on 8 December when the value exceeded those of 1 September or of 3 November to a high degree of significance.

The mean results for each sampling date in 1959 are set out in table 4. In this year weather conditions were drier than usual during the sampling period. No significant changes in porosity occurred over the period of this trial. The content of free-draining pores and the volume of water drained did not change until the last sampling. On 7 September the volume of free-draining pores was significantly greater

TABLE 4. Mean Structural Properties of Soil on Sampling Dates in 1959 (0.3–1.2 in. depth)

Date	Porosity (%)	(S.D.)	Volume of pores Drained at 50 cm of suction (%)	(S.D.)	Water drained by 50 cm of suction (%)	(S.D.)	Estimated vol. of wormholes (%)	(S.D.)	Wt. of roots in 100 ml of soil (g)	Previous Weather and Grazing Conditions
27 July	63.6	(2.7)	5.2	(1.8)	5.5	(1.4)	1.2	(0.6)	—	Soil recently grazed when wet
10 Aug.	64.0	(2.1)	5.6	(1.7)	5.3	(1.5)	—		1.10	No further grazing; moist soil
24 Aug.	63.6	(2.4)	5.1	(2.2)	5.9	(1.5)	—		1.31	No further grazing; moist soil
7 Sept.	62.4	(1.9)	6.7	(1.3)	8.0	(1.2)	1.6	(0.7)	—	No further grazing; soil drying

than on 27 July or 24 August: the volume of water drained was greater than on any of the preceding dates to a high degree of significance.

The relationship between pores found drained in the field at the time of sampling and pores drained at 50 cm of suction is illustrated in table 5. Here mean results are set out for the five occasions when the last rain had fallen between 2 hours and 2 days before a sampling.

TABLE 5. COMPARISON OF AIR-SPACE IN FIELD AND VOLUME OF PORES DRAINED BY 50 CM OF SUCTION

Sampling Date	Vol. of Pores drained at 50 cm of Suction (%)	Air-space in Field (%)
18 June 1957	5.4	5.8
30 September 1957	4.9	5.4
21 October 1957	3.1	6.0
28 July 1958	6.3	7.4
10 August 1959	5.6	5.3

It is seen that the volume of air-space found drained on this site a short time after rain approximated to the volume drained by 50 cm of suction. On 21 October 1957, when the volume drained in the field exceeded this value, conditions of high evaporation had prevailed in the 30 hours between rain and sampling.

Volumes of Wormholes and Content of Roots

Mean volumes of wormholes were between 1% and 2% of the soil volume. There did not appear to be any large changes in this quantity between dates of sampling. The mean weight of roots in 100 ml of soil did not change appreciably between dates in any given year, either.

For sampling dates on which the requisite determinations were made, the volume of wormholes or weight of roots in each soil core was plotted against the porosity of the core and the volume of water drained from it by 50 cm of suction. As was to be expected, the cores containing more wormholes tended to have higher contents of both total and free-draining pores. A strong tendency was also found for the cores containing a greater weight of roots to have higher porosities. On each of the six dates when roots were extracted the relationship between porosity and root weight was highly significant. On the average an increase of 1 g in weight of roots in 100 ml of soil was associated with an increase of 5.3% of porosity. It also appeared that the cores containing more roots lost a greater quantity of water under a suction of 50 cm of water. This relationship was less marked than the porosity one but the regressions attained significance on the two dates 11 August 1958 and 10 August 1959. On these occasions an increase of 1 g in weight of roots in 100 ml of soil was associated with increases of 1.4% and 2.9% of water drained, respectively.

Discussion

In view of the variable incidence of wet weather it could not be expected that fluctuations of soil structure would follow the same course in different years, even on the same soil and with the same intensity of set-stocking. In fact the content of free-draining pores followed in 1958 a different course from that of the preceding year. In 1957 the usually dry August was followed by an increase in this volume in September and the unusually wet October by a second fall of free-draining pores to their previous minimum value. In 1958 the wettest month was August and the content of free-draining pores decreased steadily until the end of that month. Thereafter the weather was dry throughout and, after an initial delay, the volume of free-draining pores increased steadily.

These changes in free-draining pores were studied in relation to the moisture of the soil at sampling. No consistent relationship appeared, in conformity with the finding above (P. 666) that field moisture did not affect the determinations significantly. It is possible, however, that some of the smaller decreases in porosity recorded represent a shrinkage on drying which would be reversed were a shower of rain to fall upon the pasture.

The extent of the structural changes recorded in 1958 seems worthy of note: within a few months the content of free-draining pores in the 0.3–1.2 in. depth fell from 10% to 3% of the soil volume. Such a decline in the inherent ability of the topsoil to drain would contribute to the wetness of the soil in winter. Reduced evapo-transpiration and the inability of subsoils to carry away additional water, are, apparently, not the only sources of wetness in heavily trodden pastures. Once a topsoil is puddled the greater quantity of water held in it at a given suction (table 3, column 5) would encourage severer puddling on further treading. Times when the soil was reasonably dry for grazing would become less frequent. Thus the loss of the soil's free-draining properties could be cumulative. Also, the smaller volumes of air-pores found in the topsoil after equivalent drainage point to poorer root aeration, which could adversely affect plant growth. Edmond (1958b) has shown that the structural condition of the soil can have significant effects on yields of ryegrass.

It appears that if treading under wet conditions does not continue the topsoil regains its free-draining structure but only after a certain lapse of time. The duration of this may depend on various factors, such as soil moisture, the extent of the structural breakdown and whether the pasture was appreciably thinned out by animal treading under wet conditions. In the present work statistically significant regain of free-draining pores took 1 2 months from the times when the lowest contents were recorded. Significant regain represented an increase of 1.5% or 2% of free-draining pores in the topsoil. The mechanism causing this regain is still to be investigated. It should be stressed that pasture growth may not recover at the same rate as soil structure.

It has been previously noted (Gradwell, 1956) that the visible signs of poor drainage and aeration in pasture soils do not necessarily coincide with maximum compaction. In the present work little

parallelism was seen between the changes in soil porosity and in free-draining pores. Porosity did not decline much when the soil was trodden under wet conditions but tended to decrease when it dried out. It would appear that in wet pasture, topsoil porosity is not a reliable index of the structural changes most likely to be of significance to the plant, i.e. those that affect drainage and aeration.

The lowest contents of free-draining pores recorded were 3% of the soil volume. While such a condition prevailed the 1-2% of worm-holes made a relatively important contribution. It may be doubted, however, whether such large, widely-spaced cavities are as effective in aerating the whole soil mass as would be an equal volume of narrower and more closely spaced air channels.

In evaluating the correlation between the root content and porosity of individual cores at any particular sampling it must borne in mind that the porosity figures for all cores are computed from a single value of soil specific gravity. The specific gravity of mineral soil solids is about 2.6 while that of root solids may not greatly exceed 1.0. Thus the porosity of a 100 ml core containing 1 g of roots may be overestimated by about 0.6% relative to a core with no roots. Failure to take into account the lower specific gravities of cores containing more roots could, however, account for only a small portion of the correlation observed, which amounted to 5.3% of additional porosity for every 1 g of additional roots. It is not known how much of this extra porosity resides in the roots themselves and how much represents a lower density in the soil surrounding root clumps, compared with the rest of the soil in the paddock. Kennedy and Russell (1948) also found, by comparing mean values for different treatments, that higher root contents were associated with higher measurements of porosity in topsoils under grass.

The correlations found, on some occasions, between root weight and volume of water drained by 50 cm of suction suggest that the increased porosity measured in the neighbourhood of roots may be accompanied by somewhat better drainage and aeration.

Conclusions

Marked fluctuations in the ability of the topsoil to drain can occur in high-fertility pastures trodden at varying moisture contents by grazing animals.

The loss of free-draining properties incurred by treading under wet conditions can take some time to repair, even after dry weather has returned and in the presence of vigorous pasture growth.

The changes in soil structure observed in this work seem explicable in terms of rainfall and stocking rates.

Acknowledgements

Acknowledgements are due to Mr D. B. Edmond of Grasslands Division, Department of Scientific and Industrial Research, Palmerston North, who collected the information on grazing management in these paddocks; to Mr. A. Glenday of Applied Mathematics Laboratory,

D.S.I.R., who carried out statistical analyses on the data; and also to the New Zealand Meteorological Service for the data on monthly rainfall totals reproduced in table 1.

References

Alderfer, R. B.; Robinson, R. R. 1947: Run-off from Pastures in Relation to Grazing Intensities and Soil Compaction. *J. Amer. Soc. Agron. 39:* 948–58.

Dreibelbis, F. R.; Post, F. A. 1943: Some Seasonal Changes in the Pore Space and Moisture Relationships of Woodland, Pasture and Cultivated Soils. *Proc. soil Sci. Soc. Amer. 8:* 102–8.

Edmond, D. B. 1958a: The Influence of Treading on Pasture. A Preliminary Study. *N.Z. J. agric. Res. 1:* 319–28.

———1958b: Some Effects of Soil Physical Condition on Ryegrass Growth. *Ibid. 1:* 652–9.

Gradwell, M. W. 1956: Effects of Animal Treading on a Wet Alluvial Soil Under Pasture. *Proc. N.Z Soc. soil Sci. 2:* 37–40.

———1958: An examination of the Surface-active Agent PR51 as an Accelerator of Drainage from Pasture Topsoils. *N.Z. J. agric. Res. 1:* 834–40.

Heinonen, R.; Pukkala, R. 1954: Seasonal Variation in Soil Structure. *Maataloust. aikakausk. J. Sci. Agr. Soc. Finland. 26:* 116–9.

Kennedy, W. K.; Russell, M. B. 1948: Relationship of Top Growth, Root Growth and Apparent Specific Gravity of Soil under Different Clipping Treatments of a Kentucky-Bluegrass - Wild-white-clover Pasture. *J. Amer. Soc. Agron. 40:* 535–40.

Kudelin, B. P. 1952: The Effect of Grazing on the Stability of Soil Structure, the Root System and the Yield of Perennial Herbage. *Augsne un Raza 2:* 119–46.

McIntyre, D. S. 1957: Physical Measurements of Soil Compaction under Pastures. *Ph. D. Thesis, Univ. of Wisconsin.*

N.Z. Soil Bureau 1954: General Survey of the Soils of North Island, New Zealand. *N.Z. Soil Bur. Bull.* (n.s.) 5. pp. 286.

O'Connor, K. 1956: Influences of Wheel and Foot Treading on Soils under Grasslands. *Proc. N.Z. Soc. soil Sci. 2:* 35–7.

Tanner, C. B.; Mamaril, C. P. 1959: Pasture Soil Compaction by Animal Traffic. *Agron. J. 51:* 329–30.

Taylor, N. H. 1954: The Role of Soil Science in New Zealand Problems. *Trans. roy. Soc. N.Z. 82:* 961–71.

CONTROL OF SUPERFICIAL SCALD ON GRANNY SMITH APPLES USING PRE-HARVEST SPRAYS OF DIPHENYLAMINE

By C. A. S. Padfield, Fruit Research Division, Department of Scientific and Industrial Research, Havelock North, and
R. M. Smock,* Pomology Department, Cornell University, Ithaca, New York

(*Received for publication, 3 May 1960*)

Summary

Pre-harvest spraying of trees with 500 – 1500 p.p.m. diphenylamine (DPA) in aqueous suspension, prepared either from a concentrated alcoholic solution or from a wettable powder, reduced subsequent development of superficial scald on Granny Smith apples in cool-store. Tree spraying was less effective than post-harvest treatment with DPA.

Introduction

Smock (1955) first reported the value of post-harvest treatment of apple fruits with diphenylamine (DPA) to control development of superficial scald in cool store. Application of DPA to fruit after pickling presents several problems and it was considered that pre-harvest sprays might be a cheap and practical method of application. Experiments designed to test this possibility are reported in this paper.

Experimental Procedure

Experiment 1

This experiment was designed to test the effect of delay between spray treatment and harvesting. Thirty mature Granny Smith trees in a commercial orchard, each estimated to be carrying up to 20 packed bushel cases of fruit, were used for the experiment. As scald is more severe on immature fruit, treatments were applied on 8 April, approximately 14 days before normal picking date to ensure that scald developed on untreated samples. The first samples were picked on the day of spraying. Each treatment was applied to 10 trees with a hand lance at a pressure of 300 lb/in.^{2}, using approximately 10 gal of spray per tree. Treatments were:

(1) Check—no treatment;

(2) DPA 500 ppm, 2.5% isopropyl alcohol plus 0.05% "Tween 20" wetting agent;

(3) DPA 1,000 ppm, 5.0% isopropyl alcohol plus 0.05% "Tween 20".

A sample of one loose bushel case of fruit was picked from each check tree and each sprayed tree on the day of spraying, and similar samples were taken on each of the three subsequent days. Samples were selected

* Professor Smock was a Fulbright Scholar in New Zealand from March to September 1958.

from all parts of the tree. A further loose bushel sample was taken from each of the 10 check trees on the day of spraying and all fruits in these cases were wrapped in oiled paper wraps before storage. Although an attempt was made to pick uniform sized fruit, the number of apples per box ranged from 75 to 125, the average being about 85. Rain occurred during harvesting on the third day after spraying, some trees of both sprayed lots being picked wet. Fruit was stored at 31°F on the day of picking. All samples were removed from storage on August 4 and then held for two weeks at between 60–70°F before examination.

Experiment 2

As no information was available on suitable formulations of DPA for tree spraying two preparations were compared in this experiment. Each of the four treatments was applied to 10 young Granny Smith trees selected randomly in a block of 40 trees. Details of treatments were as follows:

(1) Check—no treatment;

(2) DPA 1,000 ppm, 2.5% isopropyl alcohol plus 0.5% "Tween 20";

(3) DPA 1,000 ppm, wettable powder (25% active ingredient);

(4) DPA 1,000 ppm, wettable powder 25% plus 0.05% "Tween 20".

Treatments were applied as sprays on 24 April. As the trees were carrying a relatively light crop of variable sized fruit it was not possible to pick individual tree samples of uniform size. Instead the whole crop of 10 trees was mixed, and 10 loose bushel cases were selected at random as the sample for that treatment. Excessive residue was left by wettable powder sprays, and to remove this, half of each sample was run through a spiral roller fruit sizing machine before storage. Half samples were recorded separately as it seemed likely that removal of residues might affect scald control. Fruit was stored at 31°F on the day of spraying and examined on 14 October after being held for 14 days at 60–70°F immediately before examination.

Experiment 3

As results from the first two experiments were promising, a modified form of Experiment 1 was applied to the same block of trees in the following season. Tree numbers were reduced to four per plot, and treatments were as follows:

(1) Control—no treatment;

(2) DPA 1,000 ppm, commercial flake, 5.0% isopropyl alcohol+ 0.075% "Tween 20";

(3) DPA 1,500 ppm, commercial flake 7.5% isopropyl alcohol+ 0.075% "Tween 20";

(4) DPA 1,500 ppm, wettable powder. Samples were picked on the day of spraying (20 April), and further samples one and three days later. This coincided with the commencement of commercial harvest. Fruit was removed from cool store on 6 November and examined 14 days later.

Results

Individual tree records were examined for possible variations in scald incidence caused by factors other than DPA treatment. No relationship between tree vigour and scald incidence was found, and none of the trees used showed consistently high or low scald susceptibility. Finally there were no consistent differences in scald incidence between trees picked before and after rain on the final harvesting day of Experiment 1. Therefor it was thought unnecessary to present detailed tree records and mean percentages per treatment of slight and severe scald are given in Tables 1, 2, and 3. "Slight" and "severe" scald were separated on the basis of area of scalded tissue. If this was less than about 2 sq. cm. an apple was graded as "slight"; if more, as "severe"—an arbitrary division based on trade practice in separating saleable from waste fruit.

TABLE 1. Incidence of Superficial Scald on Granny Smith Apples from Trees Sprayed with DPA Suspensions Prior to Harvest

Treatment	Days between spraying and picking	Superficial scald		Sunburn scald %
		Slight %	Severe %	
Control	0	38	44	2
500 ppm DPA	0	13	5	7
1,000 ppm DPA	0	7	2	6
Oiled wraps	0	25	5	1
Control	1	40	38	1
500 ppm DPA	1	10	1	1
1,000 ppm DPA	1	4	1	4
Control	2	43	38	1
500 ppm DPA	2	13	1	3
1,000 ppm DPA	2	6	1	5
Control	3	34	48	1
500 ppm DPA	3	15	2	5
1,000 ppm DPA	3	14	4	2

Sprayed—8 April 1958
Harvested and Stored—8, 9, 10, 11 April 1958
Examined—18 August 1958

The tables show that pre-harvest tree spraying with DPA reduced but did not eliminate scald. In general pre-harvest spraying with DPA gave as good or better control of scald than did oiled wraps. A delay of three days between treatment and harvest had no consistent effect on scald control, either between treatments or between seasons. Mechanical removal of excess DPA deposit left by sprays of wettable powder did not impair scald control. Sunburn scald (Table 1) was not controlled by DPA; in fact, there is a suggestion of more of this type of injury following DPA application, but this is probably due to superficial scald on control samples masking sunburn scald.

TABLE 2. Incidence of Superficial Scald on Granny Smith Apples from Trees Sprayed with Three Formulations of Diphenylamine. Some Samples Mechanically Cleaned

Treatment	Superficial Scald Slight %	Scald Severe %
Check	37	32
1,000 ppm from alcohol solution	0.5	0
1,000 ppm D.PA from alcohol solution. Fruit mechanically cleaned	1	0
1,000 ppm DPA from wettable powder 25%	0	0
1,000 ppm DPA from wettable powder 25%. Fruit mechanically cleaned	0	0
1,000 ppm DPA from wettable powder 25% + 0.05% tween 20	0	0
1,000 ppm DPA from wettable powder 25% + 0.05% tween 20. Fruit mechanically cleaned	0.2	0

Sprayed, harvested, cleaned and stored—24 April 1958
Examined—October 14 1958.

Sunburn scald is a black discolouration of the skin that is not present at harvest, but which subsequently develops on certain parts of Granny Smith apples in cool store. Susceptible areas seem to be those which have been turned golden through excessive exposure to sunlight during the latter part of the growing period. Lesions have an irregular outline similar to true superficial scald, but they are much darker in colour and not depressed. Superficial scald may develop on any part of the skin whether sunburnt or not, but sunburn scald is confined to sunburned areas.

DPA wettable powder caused slight damage to apples particularly in the stalk cavity where deposits tended to concentrate. This took the form of irregular darkened patches resembling scald.

Conclusion

In an earlier paper (Padfield, 1959) it was shown that complete scald control on Granny Smith apples can be obtained using post-harvest applications of DPA. As pre-harvest sprays allowed an appreciable percentage of scald to develop they cannot be recommended in their present form for New Zealand fruit intended for long storage.

Acknowledgments

The authors gratefully acknowledge the help of Mr K. Spackman,

TABLE 3. Incidence of Superficial Scald on Granny Smith Apples from Trees Sprayed with Diphenylamine

Treatment	Days between spraying and picking	Superficial scald		Damage
		Slight %	Severe %	
Control	0	11	87	
1,000 ppm DPA from alcohol solution	0	31	2	
1,500 ppm from alcohol solution	0	19	2	
1,500 ppm DPA from wettable powder	0	14	6	2
Oiled wraps	0	27	4	
Control	1	28	69	
1,000 ppm DPA from alcohol solution	1	46	2	
1,500 ppm DPA from alcohol solution	1	23	0	
1,500 ppm DPA from wettable powder	1	12	0	12
Control	3	35	58	
1,000 ppm DPA from alcohol solution	3	38	11	
1,500 ppm DPA from alcohol solution	3	18	0	
1,500 ppm DPA from wettable powder	3	14	0	26

Harvested and stored—20, 21, 23 April 1959
Sprayed—20 April 1959
Examined November 20 1959

orchardist of Hastings, who provided trees for this project; and Mr G. G. Taylor of the Fruitgrowers Chemical Company, Mapua, Nelson, for formulating the DPA wettable powder.

References

Padfield, C. A. S. 1959: *N.Z. J. agric. Res. 2:* 953–70.
Smock, R. M. 1955: *Amer. Fruit Grower 75:* 20.

EVALUATION OF GENERAL AND SPECIFIC COMBINING ABILITY IN A LATE-FLOWERING VARIETY OF RED CLOVER (*Trifolium pratense* L.)

By L. B. Anderson, Grasslands Division, Department of Scientific and Industrial Research, Palmerston North

(*Received for publication, 13 July 1960*)

Summary

General combining ability (G.C.A.) and specific combining ability (S.C.A.) were determined for several characters in diallel crosses of seven non-inbred plants of a late-flowering variety of red clover.

Significant differences in G.C.A. and S.C.A. between the seven parents were found for yield in spring, summer and autumn.

Significant differences in G.C.A. and S.C.A. for habit, persistence and flowering were also found.

Estimates of the variances of G.C.A. and S.C.A. for the seven parents showed that S.C.A. variances were generally higher than G.C.A. variances for visual estimates of yield for these seasons, but for the other characters studied estimates of G.C.A. variances were generally greater than S.C.A. variances.

There was also some indication that both G.C.A. and S.C.A. interacted with seasons.

Introduction

A knowledge of the combining ability of superior plants of pasture species becomes increasingly important when the breeder considers the basic plants he will use in building a synthetic variety. Several procedures are in common use to test combining ability, including top crosses, open-pollination, dialled crosses, and polycrosses, as indicated by Frandsen (1952), Hanson and Carnahan (1956), and Morrow *et al.* (1958).

Torrie *et al.* (1952) used the polycross test in studying the effects of sibbing on forage yield and combining ability in red clover, and found differences in general combining ability among the sib lines.

Torrie (1957), who determined general combining ability (G.C.A.) and specific combining ability (S.C.A.) from diallel crosses in perennial ryegrass, stated that the best theoretical estimates of combining ability are obtained by the diallel system as it is a set of all possible crosses within a group of plants; but that its use, however, is limited to a small number of plants.

Sprague and Tatum (1942) in defining G.C.A. and S.C.A. have defined G.C.A. as "the average performance of a line in hybrid combinations", and state that it is due to the additive portion of genetic variance. S.C.A. "the deviation from expectation on the basis of average preformance", is due to dominance and epistasis or non-additive genetic variance.

Rojas and Sprague (1952) found this classification of S.C.A. to be inadequate and suggested the inclusion of genotype × environmental interactions in S.C.A. effects.

In the present study to assess the value of a group of later-flowering red clover plants for further breeding purposes, the diallel cross was used to determine G.C.A. and S.C.A. for visual estimates of yield and other characters of agronomic importance.

MATERIAL AND METHODS

Seven non-inbred parents, designated A–G, studied in this investigation, were from a population of late-flowering red clover used in the plant improvement programme at the Grasslands Division, Palmerston North. All, with the exception of plant G, had undergone some form of progeny test previously. Parent F was virus infected. This had a detrimental effect on seed setting in crosses with this plant. As a result, one cross F × B and its reciprocal B × F was not made. The virus infection had no obvious effect on the subsequent performance of progeny from other crosses with plant F. Progenies from diallel crosses of these seven parents were compared for various characters.

Progenies were sown in boxes in the glasshouse and then transferred to the field at the end of August 1956 using a 2-ft spacing. The design was a 5 × 5 balanced lattice of six replications (this layout also included progenies obtained by open-pollination of these plants in an isolation cage). The progenies were placed in plots of two rows of 10 plants each, one row of a cross and one of its reciprocal. Visual estimates of yield were made on six occasions from 6 December 1956 to 29 April 1958 on a 0–5 scale. The plants were grazed by sheep soon after each note was taken, and also at other times as required to control growth. The period of regrowth before a note was made was usually from six to eight weeks. Data on habit and flowering were collected. For "habit" each plant was placed into one of the following four classes: prostrate, semi-prostrate, semi-erect, or erect. For analysis the percentage of prostrate plus semi-prostrate plants was transformed to arc sin $\sqrt{\text{percentage}}$. Notes on time of flowering in 1957 began on 25 January and continued every 2–3 days for a period of one month, plants having five heads fully open or showing pink florets being classed as flowering. Data from each plot were recorded as mean number of days after 25 January 1957. In 1958 the number of plants in flower on one day—3 February—were countered.

The ability of plants to recover after flowering in the first year was regarded as "persistence" for the purposes of this paper, and was assessed on the numbers of plants surviving on 7 May 1957.

Estimates of G.C.A. and S.C.A. effects were made using method III and model I of Griffing (1956) for a set of F1's and reciprocals but no selfings. Because the 5 × 5 balanced lattice included six lines not used in the diallel study, the diallel cross data were analysed as a randomised block design to obtain the error term to be used in the analyses or variance for combining ability.

Heritability estimates were calculated from the analyses of variance for combining ability using the formula,

$$h^2 = \frac{\sigma^2 g}{\sigma^2 g + \sigma^2 s + \sigma^2 r + \sigma^2}$$

where σ^2g = the mean square for G.C.A.
σ^2s = the mean square for S.C.A.
σ^2r = the mean square for Reciprocals
σ^2 = the error mean square
This formula determines heritability in the narrow sense.

RESULTS

The results will be presented in two main sections:

(1) Estimates of G.C.A. and S.C.A. effects for visual estimates of yield in spring, summer and autumn;
(2) Estimates of G.C.A. and S.C.A. effects for habit, persistence and flowering.

and they are also discussesd in relation to possibilities for further selection.

(1) *Estimates for Seasonal Yield*

To analyse the data on a seasonal basis combining the notes from the two years, the means of two notes were used and for the purpose of this paper were classed as spring, summer and autumn, viz.

6 December 1956 and 31 October 1957	spring
25 January 1957 and 21 January 1958	summer
7 May 1957 and 29 April 1958	autumn

The F_1 yield scores for the three seasons thus represented are shown in Table 1.

TABEL 1. YIELD SCORES FOR SPRING, SUMMER AND AUTUMN
(20 plant means, cross and reciprocal)

Cross	Spring	Summer	Autumn
A × B	56.1	53.6	40.4
A × C	52.6	47.7	35.3
A × D	43.5	50.1	42.3
A × E	48.1	56.3	44.1
A × F	49.1	51.5	42.3
A × G	43.4	39.4	23.1
B × C	43.6	53.3	31.6
B × D	46.0	53.5	29.6
B × E	43.4	54.1	36.9
B × F	45.5†	50.8†	31.0†
B × G	41.0	36.9	13.1
C × D	43.1	51.4	23.0
C × E	41.2	45.3	25.6
C × F	47.1	54.8	35.2
C × G	38.4	45.9	17.4
D × E	26.3	31.6	16.9
D × F	43.4	52.9	29.4
D × G	43.0	47.9	16.5
E × F	41.0	52.2	31.3
E × G	42.3	48.4	22.0
F × G	44.3	45.3	22.3
SE ±	2.3	3.0	3.7
Sig. Diff. at 5% level	6.6	8.4	10.5

† Missing values estimated for B × F

There were significant differences between the F_1 values at each season, as shown in the following examples: A × B had significantly higher estimates of yield, than A×D, A×E, A×F, and A×G in the spring. There were many other such differences in yield estimates between progenies. Correlation coefficients showed that there was a significant positive relationship between spring and summer yield, viz., $r = +0.661$, and that there were positive but non-significant correlations between spring and autumn $r = +0.046$ and summer and autumn $r = +0.221$.

G.C.A. and S.C.A. effects were determined and the analyses of variance indicating combining ability are shown in Table 2.

TABLE 2. ANALYSIS OF VARIANCE OF YIELD SCORE FOR SPRING, SUMMER AND AUTUMN

Source	d.f.	Mean Squares		
		Spring	Summer	Autumn
G.C.A.	6	32.71**	21.03**	118.38**
S.C.A.	13	10.12**	20.85**	12.09**
Reciprocals	20	2.35	2.04	3.09
Error	195	1.66	2.14	2.65
h^2 %		70	46	87

** $p < 0.01$

This table indicates that there were highly significant effects due to both G.C.A. and S.C.A.

There was no evidence of any reciprocal differences. Heritability estimates also shown were relatively high.

Estimates of G.C.A. effects for each parent were calculated and are shown in Table 3.

TABLE 3. ESTIMATES OF G.C.A. EFFECTS ON YIELD SCORES IN SPRING, SUMMER AND AUTUMN

	Combining Ability Effects		
	Spring	Summer	Autumn
A	2.9	0.6	5.3
B	1.2	1.0	0.9
C	0.3	0.6	— 0.6
D	— 1.8	— 0.5	— 1.6
E	— 2.1	— 0.4	0.3
F	0.7	1.5	1.7
G	— 1.1	— 2.9	— 6.0
SE ±	0.4	0.4	0.5
Sig. diff. at 5%	1.1	1.1	1.4

Estimates in the above table are contributions of the effects to the data in Table 1; a G.C.A. effect of +2.9 is the mean contribution of parent A to all 20 plant notes involving that plant. These effects are deviations about a zero mean.

A comparison of the relative performance of the seven parents in spring, summer and autumn can be made from this table.

Parent A had significantly higher positive effects in spring and autumn than any other parent, but in summer this was not so. Parent F was a relatively good combiner, being better than D, E, and G in spring and summer, and C, D, and G in the autumn.

Estimates of S.C.A. effects were calculated and are shown in Table 4.

TABLE 4. ESTIMATES OF S.C.A. EFFECTS ON YIELD SCORES IN SPRING, SUMMER AND AUTUMN

Season	Parent	Parent					
		B	C	D	E	F	G
Spring	A	2.0	1.2	—1.3	1.3	—1.0	—2.1
	B		—1.6	1.7	0.7	—1.0	—1.6
SE ± 0.7	C			1.2	0.5	0.7	—1.9
Sig. diff. at	D				—4.9	0.9	2.5
5% 2.1	E					0.0	2.4
	F						0.6
Summer	A	0.8	—1.8	0.6	3.6	—0.8	—2.4
	B		0.7	1.9	2.1	—1.5	—4.1
SE ± 0.8	C			1.2	—1.9	0.9	0.8
Sig. diff. at	D				—7.6	1.2	2.9
5% 2.4	E					0.7	3.1
	F						—0.4
Autumn	A	—0.5	—1.6	2.9	1.9	—0.4	—2.3
	B		1.0	1.1	2.8	—1.6	—2.8
SE ± 0.9	C			—0.8	—1.4	2.0	0.8
Sig. diff. at	D				—4.7	0.1	1.4
5% 2.6	E					—0.9	2.2
	F						0.9

There were many significant differences in estimates of S.C.A. effects between F_1's within seasons, such as those for A×B which had significantly higher positive effects than A×D, A×F and A×G in the spring. In summer A×B had significantly higher positive effects than A×C and A×G, but in autumn A×D had significantly higher positive effects than A×B. There were other such interactions with season.

A more direct comparison of G.C.A. and S.C.A. effects for each parent was made by calculating estimates of C.G.A. and S.C.A. variances (Griffing, 1956). These are shown in Table 5.

Estimates of S.C.A. variances were higher than G.C.A. variances for parents B, C, D and E at each season. For parent F, estimates of C.G.A.

TABLE 5. ESTIMATES OF G.C.A. AND S.C.A. VARIANCES FOR SEASONAL YIELD

Parent	Season	S^2g	S^2s
A	Spring	8.3	1.5
	Summer	0.0	3.0
	Autumn	28.1	2.1
B	Spring	1.2	1.3
	Summer	0.6	3.9
	Autumn	0.3	2.1
C	Spring	— 0.2	0.6
	Summer	0.0	0.3
	Autumn	— 0.1	0.0
D	Spring	3.0	5.4
	Summer	— 0.1	13.0
	Autumn	2.2	4.6
E	Spring	4.2	5.1
	Summer	— 0.2	16.2
	Autumn	— 0.4	6.1
F	Spring	0.2	— 0.6
	Summer	1.9	— 0.6
	Autumn	2.6	— 0.5
G	Spring	0.9	3.2
	Summer	7.8	6.6
	Autumn	37.1	2.1

variance were higher than S.C.A. variances at each season. Parent A showed an interaction of variance estimates of G.C.A. and S.C.A. in that G.C.A. was greater in spring and autumn whereas S.C.A. was greater in the summer. Parent G on the other hand had higher estimates of S.C.A. variance in the spring, while G.C.A. variance was more important in the summer and autumn. The very high estimates of G.C.A. variances for parents A and G in the autumn is most likely due to differences in persistence. This factor would also influence the higher heritability estimates in the autumn.

(2) *Habit, Persistence and Flowering*

In this study "habit" is represented by the percentage of prostrate or semi-prostrate plants in the popualtion. The methods of estimating flowering time and persistence were described earlier.

Table 6 shows the analyses of variance indicating combining ability for these characters.

As in yield there were highly significant effects due to both G.C.A. and S.C.A. Estimates for heritability shown at the bottom of Table 6 are higher than those for yield with the exception of the heritability estimates for yield in autumn.

TABLE 6. Combining Ability Analyses of Variance for Habit, Persistence and Flowering

Source	d.f.	Mean Squares			
		Habit	Persistence	Flowering Date 1957	Flowering Percentage 1958
G.C.A.	6	1210.00**	233.02**	144.37**	896.33**
S.C.A.	13	91.78**	28.87**	10.73**	68.50**
Reciprocals	20	26.29	6.21	0.91	29.60
Error	195	23.11	10.17	4.94	27.48
h^2 %		90	84	90	88

** $p < 0.01$

Estimates of G.C.A. effects for each parent for habit, persistence and flowering are shown in Table 7.

TABLE 7. ESTIMATES OF G.C.A. EFFECTS ON HABIT, PERSISTENCE AND FLOWERING

Parent	General Combining Ability Effects			
	Habit 1956	Persistence 1957	Flowering Date 1957	Flowering Percentage 1958
A	— 1.4	7.2	2.9	— 11.7
B	16.7	— 2.5	— 3.9	8.4
C	— 14.1	— 5.9	6.0	— 14.4
D	— 5.8	0.6	0.2	0.0
E	— 5.8	4.0	1.8	2.9
F	— 2.8	1.7	— 3.5	5.3
G	13.1	— 5.2	— 3.4	9.3
SE ±	1.4	0.9	0.7	1.5
Sig. diff. at 5%	4.0	2.6	1.8	4.3

Parents B and G with high positive effects for habit have a greater number of prostrate and semi-prostrate plants in their F_1's, while parents C, D and E with high negative effects have a greater proportion of erect and semi-erect plants in their F_1's.

A and E were superior combiners for persistence while C and G were inferior.

In 1957 parents B, F and G significantly increased earliness of flowering while parents A and C significantly delayed flowering. The pattern was similar for flowering percentage in 1958 (negative figures in the last column of Table 7 indicate late flowering). B, F and G again increased earliness while A and C delayed flowering.

Estimates of S.C.A. effects for the characters being discussed are shown in Table 8.

There were many sifinificant differences in effects between the F_1's for all characters, some examples being the following: A×B had significantly less prostrate plants than A×D, A×E or A×G; showed less persistence than A×D, and A×G, and was later in time of flowering than A×D or A×G. There were many other differences in effects. Inconsistencies in S.C.A. effects on flowering between 1957 and 1958 may have been due to the difference in the system of note taking in these years.

Table 9 shows the estimates of G.C.A. and S.C.A. variances for habit, persistence and flowering.

For these characters estimates of G.C.A. variances were generally much higher than S.C.A. variances. Some exceptions were parents A and F which had higher S.C.A. variances for habit, and parents B and D which had higher S.C.A. variances for persistence.

TABLE 8. ESTIMATES OF S.C.A. EFFECTS ON HABIT, PERSISTENCE AND FLOWERING

Parent	Character	Parent					
		B	C	D	E	F	G
A	Habit	—5.6	—5.0	4.2	5.2	—7.4	8.5
	Persistence	—3.8	1.8	3.0	—0.4	—2.6	2.1
	Flowering Date 1957	—3.1	1.0	1.9	—0.6	—1.1	1.9
	Flowering Percentage 1958	1.9	6.7	—1.7	—4.1	1.0	—4.0
B	Habit		3.0	4.6	3.7	—8.3	2.6
	Persistence		1.0	1.6	6.7	0.6	—6.2
	Flowering Date 1957		0.4	—1.2	—0.6	4.3	0.1
	Flowering Percentage 1958		—3.9	0.2	7.8	—8.1	1.9
C	Habit			—0.3	3.0	3.0	—3.8
	Persistence			—2.3	—0.3	—0.6	0.4
	Flowering Date 1957			2.2	—0.2	—1.5	—2.2
	Flowering Percentage 1958			—1.5	1.1	3.3	—5.8
D	Habit				—5.6	2.3	—5.2
	Persistence				—6.8	2.5	1.9
	Flowering Date 1957				0.7	—0.8	—2.9
	Flowering Percentage 1958				—7.3	0.3	9.8
E	Habit					3.1	—9.4
	Persistence					—0.5	1.2
	Flowering Date 1957					—1.9	2.1
	Flowering Percentage 1958					3.9	—1.6
F	Habit						7.3
	Persistence						0.6
	Flowering Date 1957						1.0
	Flowering Percentage 1958						—0.5

	Habit	Persistence	1957 Flowering Date	1958 Flowering Percentage
SE ±	2.8	1.8	1.3	3.0
Sig. diff. at 5%	7.8	5.2	4.6	8.5

DISCUSSION

In selecting basic plant material for use in building a synthetic variety of a pasture species or forage crop, G.C.A. is generally considered to be of more importance than S.C.A. Morley (1958) has pointed out

TABLE 9. ESTIMATES OF G.C.A. AND S.C.A. VARIANCES FOR PERSISTENCE AND FLOWERING

	Habit		Persistence		Flowering Date 1957		Flowering Percentage 1958	
	S^2g	S^2s	S^2g	S^2s	S^2g	S^2s	S^2g	S^2s
A	—2.1	27.3	50.4	—0.6	7.3	—0.1	131.5	—4.9
B	275.6	11.8	4.6	12.2	14.2	2.0	66.4	7.8
C	195.7	—5.1	33.0	—6.2	34.9	—1.3	201.8	—0.4
D	29.1	2.2	—1.4	6.2	—0.8	—0.1	—4.7	8.9
E	29.8	17.5	14.5	10.3	2.5	—2.2	3.8	8.0
F	3.7	21.5	0.2	—5.3	11.5	1.5	23.6	—3.4
G	166.6	34.0	25.2	—1.4	11.0	0.5	82.3	8.4

that in segregating material when G.C.A. variance is high relative to the other fractions, results of crosses may be predicted with reasonable precision, enabling more rapid progress in selection. This may not be the case if selection for high S.C.A. variance is carried out. Kalton and Leffel (1955) have suggested that if selection for high S.C.A. variance is practised, the performance of a synthetic variety may deteriorate in the second or later generations of seed increase due to the dissipation of non-additive effects. Torrie (1957) in studying two populations of perennial ryegrass, one previously selected and the other unselected, found that S.C.A. estimates were generally greater than G.C.A. estimates in the selected population. The reverse was the case in the unselected population. In cocksfoot (*Dactylis glomerata*), Kalton and Leffel (1955) also found that G.C.A. was more important in unselected populations. Sprague and Tatum (1942) have suggested that this is because the selected plants are more similar to each other in performance than plants in an unselected population, and differences in additive effects are largely eliminated, allowing non-additive interactions to become more important.

Certain conclusions can therefore be drawn about the material studied in the present experiment. For yield scores, estimates of S.C.A. variances were generally higher than G.C.A. variances, with the exception of parent F, and parents A and G in some seasons. Thus the parents used in this experiment, as far as yield scores are concerned, generally followed the pattern for selected populations. The relatively high heritability estimates indicate that improvements in yield should be possible in all seasons. For the other characters studied—habit, persistence and time of flowering—G.C.A. variances were generally higher than S.C.A. variances. There were some exceptions, but as selection for these characters had not previously been carried out with the exception of some selection for persistence in a wider sense than used in this trial, the high G.C.A. variances were not unexpected. The high heritability estimates for habit, persistence and flowering time indicate that further improvement would be possible, and as they are characters of agronomic importance, selection for them should be a part of any future breeding programme.

The prostrate habit has been considered to be typical of Montgomery red clover, a late flowering ecotype from Montgomeryshire in Wales. If this is the ideal type for a later flowering red clover, selection towards a dense prostrate or semi-prostrate type of plant would be desirable, but would have some limitations within the parents studied in this trial. Parents B and G have significant G.C.A. effects for prostrate habit while parent C had significant effects for erectness of habit. Parents A, D, E and F were intermediate in their effects with D and E tending towards erectness of habit.

Selection for persistence is an integral part of any breeding programme in perennial or semi-perennial pasture species. Persistence as measured by the ability to recover after flowering, showed that parents A and E had the highest effects. Parents C and G were significantly poorer in persistence, while B, D and F were intermediate.

The time of flowering of parents used in a synthetic variety should be within fairly narrow limits. From the significant differences in G.C.A.

effects for time of flowering found in this material it is thought that such differences would be too great for use in a synthetic variety. The time of harvesting for seed in later generations of a variety composed of such plants may induce a shift in population structure towards early or late flowering, thereby reducing the effective number of parents comprising the variety. This may tend to produce inbreeding effects if the initial number of basic plants was relatively small. A further problem associated with selection for flowering time within this material is related to seasonal production. If autumn growth is a requirement from this material there were four plants which contributed most to positive G.C.A. effects for growth in this season. These were A, B, E and F with A and F having the highest effects. If later flowering time is also a requirement, as well as autumn production, parent A was the only one which had both these characters. B and F had significant effects for earliness of flowering time while parent E was intermediate.

In further selection from within this material there may be some difficulty in finding a sufficient number of unrelated plants for a synthetic variety based on plants which have semi-prostrate to prostrate habit, are persistent and late flowering, and have good autumn production.

Rojas and Sprague (1952) have stressed the importance of the inclusion of genotype × environment interactions in S.C.A. effects, and Jinks and Stevens (1959) found evidence of seasonal differences in the non-allelic components of variation in diallel crosses in *Nicotiana*. Evidence of such interactions of S.C.A. with seasons was found in the present trial in the performance of cross A×B which showed significantly higher positive effects for growth in the spring than autumn, and for crosses A×D and B×C which showed significantly higher positive effects in autumn than in spring.

Evidence of genotype × environmental interactions is found in G.C.A. effects for yield seasons in Table 3 where the estimates of G.C.A. effects for parent A were seen to be significantly higher in the autumn than in summer, whereas for parents B, E and F, there was no significant difference. Torrie (1957) found similar interactions of plant with season in perennial ryegrass.

Such genotype × season interactions are important in breeding perennial or semi-perennial pasture species as they enable selection to be carried out for growth in a particular season. Also, where spread of production is required, they enable selection of plants which have different seasonal peaks of growth to be utilised in building a synthetic variety.

Acknowledgment

The author wishes to thank Mr A. C. Glenday, Applied Mathematics Laboratory, for assistance with statistical analyses and interpretation of results, and Professor J. H. Torrie, University of Wisconsin, who offered useful criticism.

References

Frandsen, K. J. 1952: Theoretical Aspects of Cross-Breeding Systems for Forage Plants. *Proc. 6th int. Grassl. Congr. pp.* 306–13.

Griffing, B. 1956: Concept of General and Specific Combining Ability in Relation to Diallel Crossing Systems. *Aust. J. biol. Sci. 9:* 463–93.

Hanson, A. A.; Carnahan, H. L. 1956: Breeding Perennial Forage Grasses. *U.S.D.A. Tech. Bull. No. 1145.*

Jinks, J. L.; Stevens, J. M. 1959: The Components of Variation Among Family Means in Diallel Crosses. *Genetics 44:* 297–308.

Kalton, R. R.; Leffel, R. C. 1955. Evaluation of Combining Ability in *Dactylis glomerata* L. III General and Specific Effects. *Agron. J. 47:* 370–5.

Morley, F. H. W. 1958: The Inheritance and Ecological Significance of Seed Dormancy in Subterranean Clover (*Trifolium subterraneum* L.). *Aust. J. biol. Sci. 11:* 261–74.

Morrow, J. W.; Proudfoot, K. G.; Wright, C. E. 1958: A Comparison of Pollination Systems in Relation to Plant Evaluation in *Lolium perenne*. *Res. exp. Rec. Minist. Agric. N. Ire. 7:* 17–26.

Rojas, B. A.; Sprague, G. F. 1952: A Comparison of Variance Components in Corn Yield Trials. III General and Specific Combining ability and their interactions with locations and years. *Agron. J. 44:* 462–6.

Sprague, G. F.; Tatum, L. A. 1942: General Vs Specific Combining Ability in Single Crosses of Corn. *J. Amer. Soc. Agron. 34:* 923–32.

Torrie, J. H. 1957: Evaluation of General and Specific Combining Ability in Perennial Ryegrass *Lolium perenne* L. *N.Z. J. Sci. Tech. A38:* 1025–35.

————; Hanson, E. W.; Allison, L. J. 1952: Effects of Sibbing on the Forage Yield and Combining Ability of Red Clover. *Agron. J. 44:* 569–73.

SCALE INSECT SURVEY ON APPLES 1959-60

By Aola M. Richards, Plant Diseases Division, Department of Scientific and Industrial Research, Auckland

(*Received for publication, 10 June 1960*)

Summary

Seven scale insects are recorded from apples in New Zealand, five of them causing red marks on fruit. They are San José scale (*Quadraspidiotus perniciosus* (Comst.)), oyster shell scale (*Q. ostreaeformis* (Curtis)), mussel scale (*Lepidosaphes ulmi* (L.)), red scale (*Aonidiella aurantii* (Mask.)), *Parlatoria pittospori* Mask., *P. virescens* Mask. of McKenzie, and greedy scale (*Aspidiotus rapax* (Comst.)). Oyster-shell scale is recorded from New Zealand for the first time. Red scale is recorded here for the first time causing red marks on apples. *P. virescens* is recorded for the first time on fruit. San José is the most important economically of these scales because it is most widespread and produces three generations each season.

Introduction

For many years scale insects have been recorded throughout New Zealand on various varieties of fruit trees. They occur on wood, leaves and fruit, in many cases causing pitting, which may be accompanied by a bright red mark. Today all export lines of fruit are required to be without blemishes, so that the prevention of pitting and red marks has become of vital importance.

During the 1959–60 season, a scale insect survey was carried out on apple trees throughout the country, more particularly in the main apple-growing districts, to determine the extent of the damage done to fruit. Infested orchards were visited in the Auckland, Nelson and Christchurch districts. For much of the survey the author is indebted to the assistance of officers in the Department of Agriculture for collecting and sending in samples of twigs and fruit for examination. A total of 35 samples have been examined from 91 different orchards. From these samples seven different scale species have been identified on wood and fruit (Table 1), five out of the seven causing red marks surrounding the insects on fruit. Those scale usually causing red markings are San José scale (*Quadraspidiotus perniciosus* (Comst.)), oyster-shell scale (*Q. ostreaeformis* (Curtis)), a new record from New Zealand, mussel scale (*Lepidosaphes ulmi*

TABLE 1. Presence of Scales on Apples in 91 Orchards Examined

Scale Species	Number of Orchards
San José	68
Oyster Shell	16
Greedy Scale	14
Mussel Scale	14
Parlatoria pittospori	5
Red Scale	2
Parlatoria virescens	1

(L.)), red scale (*Aonidiella aurantii* (Mask.)) and *Parlatoria pittospori* Mask. The scales occurring without making red marks are greedy scale (*Aspidiotus rapax* (Comst.)) and *Parlatoria virescens* Mask. of McKenzie 1945, a new record from New Zealand on fruit. The intensity of the red mark varies markedly from species to species ranging from an orange red in oyster-shell scale, through bright red in San José, mussel scale and red scale to a dark red in *P. pittospori.*

Of the seven scales the most important economically are San José, *Parlatoria pittospori,* oyster shell and mussel shell. *P. virescens* and red scale have been recorded only from Auckland. Although red scale is well known as a pest on apple trees overseas, this appears to be a new record for New Zealand. Greedy scale has a wide geographic distribution, but occurs very sporadically and is of minor importance.

The distribution of these species is of interest (Table 2). San José scale and *P. pittospori* occur in similar districts in the North Island and northern part of the South Island. Mussel scale occurs throughout the country, particularly in neglected orchards. In Canterbury and Central Otago it is more particularly associated with oyster-shell scale in both neglected and commercial orchards.

TABLE 2. DISTRICTS IN WHICH SCALES OCCUR ON APPLES IN NEW ZEALAND

San José	*Oyster-Shell*	*Greedy Scale*	*Mussel Scale*	*Red Scale*	*Parlatoria pittospori*	*Parlatoria virescens*
Auckland	Christchurch	Auckland	Auckland	Auckland	Auckland	Auckland
Hamilton	Central Otago	Nelson	Hawkes Bay		Hawkes Bay	
Thames	Oamaru	Christchurch	Wellington		Marlb'r'gh.	
Opotiki			Nelson		Nelson	
Otakiri (Tauranga)			Christchurch			
Gisborne			Central Otago			
Hawkes Bay						
Greytown						
Marlb'r'gh.						
Nelson						

San José is the most important species because, in New Zealand, it passes through three generations each season and a single adult female is capable of producing up to 1,400 young. The first-generation crawlers appear in October and go straight on to the newly formed fruit. The second generation appears in late January and early February, and is well established before commencement of harvesting. The third generation appears in early April and does damage to late season fruits. This final generation then overwinters till the next spring. Each generation marks and pits wood and fruit. Red marks may be formed within 24 hours of crawlers settling.

South of Nelson and Marlborough San José scale does not appear to occur, and its place is taken by the oyster-shell scale. This is in agreement with findings in other parts of the world, more particularly in Austria (Fulmek, 1941) where San José scale occurs in warm low-lying districts and is replaced by oyster-shell scale in more exposed situations. According to overseas workers (Fulmek, 1941; Lasaroff, 1940) oyster-shell scale has but one generation each season. This appears to be true for New Zealand. Adults mature throughout the spring and early summer, followed by prolific crawler release. Unlike San José scale which gives birth to living young, oyster-shell scale produces eggs. Although hundreds of crawlers may settle on fruit, forming thick incrustations, they never pass beyond the second nymphal instar and they do not produce the large number of pits and red marks associated with San José scale.

Parlatoria pittospori Mask. occurs only on fruit. Its life cycle is passed on pine trees, many of which form shelterbelts to orchards. From there crawlers may be blown onto fruit. These crawlers may become established on the fruit producing a second generation there. Once settled, this scale usually produces characteristic dark red marks on fruit. As its distribution in orchards is usually limited to areas near pine trees, it is not as economically important as San José scale.

Most intensive sampling has been carried out in the Auckland district, and Table 3 shows the localities and number of orchards from which the different scales were recorded.

TABLE 3. DISTRICTS NEAR AUCKLAND WHERE SCALES ON APPLES WERE COLLECTED

District	San José	Greedy Scale	Mussel Scale	Red Scale	*Parlatoria pittospori*	*Parlatoria virescens*
Albany	1					
Avondale	1					
Glen Eden	1					
Helensville	1	1†				
Henderson	13 – 1†			1	1	1†
Huapai	8	1†			2	
Mt Albert	2	1†	1†			
Oratia	7	7†		1		
Port Albert		2†				
Ranui	1†					
Te Hana					2	

Figures indicate number of orchards

† indicates no red mark present

To assist in the identification of these scales, the following is a key to the scale insects recorded from apple trees in New Zealand during this survey:

HAND LENS KEY TO SCALE INSECTS ON APPLE TREES

1. Scales of females elongate 2
   Scales of females round 3

2. Scales of females at least twice as long as wide and up to 3 mm in length ..... **Lepidosaphes ulmi** (L.)
   Scales of females less than twice as long as wide and up to 1.5 mm in length. Body mauve ..... **Genus Parlatoria** Targioni

3. Scale of female flat, exuviae central, red-brown in colour ..... **Aonidiella aurantii** (Mask.)
   Scale of female convex, exuviae grey 4

4. Scale of female highly convex, the exuviae near one side, giving scale a strongly tipped-over appearance ..... **Aspidiotus rapax** Comst.
   Scale of female only slightly or moderately convex 5

5. Scale of female circular, slightly convex, exuviae subcentral, about 0.9 mm in length. Body bright yellow ..... **Quadraspidiotus perniciosus** (Comst.)

   Scale of female subcircular, moderately convex, exuviae subcentral, about 1.3 mm in length. Body pale yellow ..... **Quadraspidiotus ostreaeformis** (Curtis)

KEY TO SCALE INSECTS ON APPLE TREES

1. Scales of females elongate 2
   Scales of females round 4

2. Scales of females at least twice as long as wide and up to 3 mm in length ..... **Lepidosaphes ulmi** (L.)
   Scales of females less than twice as long as wide and up to 1.5 mm in length 3

3. Dorsal submarginal macroducts ranging from 35 to 68 with average of 43 on each side. Three pairs of pygidial lobes once-notched on both sides ..... **Parlatoria pittospori** Maskell
   Dorsal submarginal macroducts ranging from 18 to 36 with average of 23 on each side. Three pairs of pygidial lobes converge apically toward meson, the median pair largest, once-notched on inner margin 2 or 3 times on outer margin; 2nd and 3rd lobes inner margin straight, outer margin angled with three notches ..... **Parlatoria virescens** Maskell of McKenzie

4. Without perivulvar pores 5
   With perivulvar pores ..... **Quadraspidiotus ostreaeformis** (Curtis)

5. Pygidium with up to 3 anal lobes 6
   Pygidium with 4 anal lobes ..... **Aonidiella aurantii** (Maskell)

6. Pygidium with large anal lobes, 2nd and 3rd lobes represented by slight points ..... **Aspidiotus rapax** Comst.
   Pygidium with two pairs of lobes. 3rd lobes lacking or represented by a slight prominence ..... **Quadraspidiotus perniciosus** (Comst.)

The following is a list of apple varieties from which scales were recorded during the survey (Table 4).

In many cases rigorous spray programmes were adhered to by orchardists to control scale; but shelter belts round orchards and home gardens acted as reservoirs for reinfestation. San José scale was found from willows *Euonymus* and flowering cherries. Oyster-shell and mussel scale were observed on flowering cherries and flowering apples. *Parlatoria pittospori* was found on pines.

TABLE 4. APPLE VARIETIES ATTACKED BY SCALE

VARIETIES	SCALES
Ballarat	1, 2, 3, 4, 5, 6, 7
Cliff's Seedling	1
Cox's Orange Pippin	1, 2, 6
Delicious	1, 2, 3, 6
Red Delicious	1, 2
Dougherty	1, 6
Dunn's Favourite	2
Frimley Beauty	1
Giant Geniton	1
Golden Delicious	1
Granny Smith	1, 3, 4
Gravenstein	1
Jonathan	1, 2, 3
Kidd's Orange Red	1, 6
Mobb's Royal	1
Oratia Special	1
Red Stateman	1, 6
Rokewood	2, 3
Rome Beauty	1
Salome	1
Stayman Winesap	1
Sturmer Pippin	1, 2, 3
Washington	1, 6
Worcester Pearmain	2, 3

KEY TO SCALES	
San José	1
Oyster Shell	2
Mussel	3
Greedy	4
Red	5
Parlatoria pittospori	6
P. virescens	7

Predatory mites were found in a number of orchards, more particularly in Christchurch and Auckland. Hymenopterous parasites of scales were also present, and in the Auckland district the ectoparasite *Aphytis diaspidis* Howard, the comman chalcid, and the endoparasite *Aspidiotiphagus citrinus* (Craw.) were identified from San José scale. Ladybird larvae were recorded from Christchurch, but were not reared, and no adults were collected. Extensive spraying of orchards greatly reduces the effectiveness of these predators.

Conclusions

Up till recently, most red spots of insect origin found on apples in New Zealand were thought to be caused by San José scale. As a result of this survey, five species of scales are now recorded causing red marks on fruit. Of these, San José scale is by far the most important economically because it produces three generations each season and is widespread in orchards.

It was also believed that San José scale was distributed throughout the whole country. It is now known that the main apple scale in Canterbury and Central Otago is not San José, but the closely related oyster-shell scale.

Extensive spray programmes have killed many of the natural predators of scales, and there is need for more work on the biological control aspects of this problem.

References

Fulmek L. 1941: Die eigentlichen Gefahren der San José-Schildlaus in der Ostmark. *Z. angew. Ent. 28:* 462–65.

Lasaroff, A. W. 1940: Untersuchungen über die echten Schildläuse (Diaspinae, Coccidae) der Obstbäume in Bulgarien. Separate, 46 pp. Sofia. (Abstr. in *Z. Pfikrankh 52* pt. 7–8 p. 428. Stuttgart, 1942) Abstract only seen in Review of Applied Entomology *31*, Series A: 77, 1943.

McKenzie, H. L. 1945: A Revision of *Parlatoria* and Closely Allied Genera (Homoptera: Coccidea: Disapididae). *Microentomology, 10:* 47–121.

FUNGI OF SOME FOREST AND PASTURE SOILS

By R. H. Thornton, Soil Bureau, Department of Scientific and Industrial Research, Taita

(*Received for publication, 26 May 1960*)

Summary

The fungi occurring in two soils under forest and five soils under pasture were examined in various seasons employing the screened immersion plate method.

Greater numbers of mycelia were isolated from soils under pasture than from the same soils under forest, and from well drained soils under pasture compared with less well drained soils under pasture.

Season and local weather conditions appeared to influence fungal growth. In soils under forest *Cylindrocarpon radicicola* was predominant and Fusaria were absent, whereas in soils under pasture Fusaria were predominant, particularly *F. oxysporum*.

The soil fungal patterns for most of the pasture soils were very similar, which suggests the development of a similar environment for fungi in these soils.

Introduction

The present work is a contribution to a broad investigation of soil microorganisms being carried out in these laboratories and is a continuation of a series of investigations of active fungi in soils. This series began with the development of a technique, the screened immersion plate method, for examining qualitatively and quantitatively the distribution of actively growing fungi in soils (Thornton, 1952, 1956). This was followed by a comparison of this technique with other methods for isolating fungi from soil (Chesters and Thornton, 1956). An examination was then carried out of fungi in mixed oakwood and heath soil profiles (Thornton, 1956). The method was then applied to an investigation of modified naturally occurring tussock grassland soils (Thornton, 1958a, b) and of agriculturally developed tussock grassland soils under root crops and under pasture of introduced grasses and clovers (Thornton, 1960a, b). The present study gives results on the occurrence and distribution of fungi in soils under broadleaf forest and in soils formerly under broadleaf forest and now under pasture of introduced grasses and clovers. These studies on soil fungi have been made in the belief that in order to determine the role of fungi in soil processes the active mycelial forms need to be distinguished qualitatively and quantitatively. Although the results obtained and the interpretations made will be largely influenced by the method employed, in the current series of investigations the active soil fungi have been studied with the same method. A summary of these investigations has already been given (Thornton, 1960c).

Earlier it was shown (Thornton, 1958b, 1960b) that the predominant fungi in fescue tussock grassland soils were non-sporing forms, the greatest numbers of isolations being of *Rhizoctonia* sp., and that the microflora

did not alter greatly when the tussock cover had been replaced by root crops or by pasture of introduced grasses and clovers. The lack of change was thought to be due to a lack of appreciable change in the type and supply of organic materials being added to the soil. In the present investigations fungi were examined in soils under forest and soils formerly under forest and now under pasture. The replacement of the forest by grass would have involved a marked change in the type and supply of organic materials available to fungi. In the forest soils there are large quantities of organic materials occurring on the surface in the form of litter composed of leaves, twigs and branches, with dead root material in the soil. The litter will have passed through various stages of degradation before being subject to attack by fungi in mineral soil layers. In soils under pasture most of the organic additions will be of root origin, with some dead leaves and dung and urine added to the surface. The supply and chemical composition of the two types of organic materials is likely to be quite different. The investigation was planned to determine the fungal populations under the two types of vegetations, to relate them to the changes which had occurred, and to compare them with results obtained in other investigations in this series. In addition to parallel forest-pasture observations, other soils formerly under forest and now under pasture, were also examined.

Materials and Methods

Modified screened immersion plates (Thornton, 1956) were employed to isolate fungi from soil. Isolations were made from the A horizon at a depth of 2 3 in. by the method previously described (Thornton, 1952). In Horotiu soil isolations were made from a depth of $1\frac{1}{2}$ in. The isolating medium was 2% distilled water agar and mycelial growths were initially grown on acidified potato-glucose agar for subsequent identification. One plate was employed in each of 10 sample pits, samples being taken at various seasons in different years. Details of sampling for each soil will be given later. Plates were buried for seven days in spring and autumn, and four days in summer.

Soils and Sites

The soils studied and their locations are given below.

(a) *Kiwitea silt loam* was formed from greywacke alluvium mixed with rhyolitic volcanic ash under mixed podocarp forest on flat-to-undulating ground and is classified as a yellow-brown loam (N.Z. Soil Bureau, 1954, p. 275). A regrowth remnant of the original forest dominated by *Beilschmieida tawa* occurs at Greystoke Reserve adjacent to Mr R. Stafford's farm, Jefferson's Line, near Marton (Map ref. N.143, 935771). Sample sites in this reserve were examined together with sample sites in a pasture adjacent to this reserve in which ryegrass (*Lolium perenne*), white clover (*Trifolium repens*) and cocksfoot (*Dactylis glomerata*) were predominant. The area now under pasture had been cleared of forest over 100 years ago, probably by burning, and after a period under scrub, was ploughed and sown with introduced pasture plants about 90 years ago.

(b) *Korokoro silt loam* was formed from greywacke under broadleaf-podocarp forest and is classified as a yellow-brown earth (Taylor and Cox, 1956). Ten sample sites were employed and were on Mr R. Bradey's farm

at Judgeford, near Wellington (Map ref. N.160, 460400). This site was cleared of forest by felling and burning and was sown with introduced pasture plants about 90 years ago. The area has never been ploughed or cultivated and is now covered mainly by browntop (*Agrostis tenuis*) and chewings fescue (*Festuca rubra* var. *fallax*).

(c) *Gollans silt loam* was formed from greywacke colluvium under coastal forest and is classified as a gley soil. An area of regrowth remnant of the original forest occurs on Mr H. Hadfield's farm at Paraparaumu, near Wellington (Map ref. N.157, 560683). Adjacent to this forest reserve is a field under pasture mainly of cocksfoot, ryegrass and clover which was cleared of coastal forest about 60 years ago and sown with introduced pasture plants.

(d) *Tokomaru silt loam* was formed from greywacke alluvium under broadleaf-podocarp forest and is classified as a yellow-grey earth. The forest was cleared over 100 years ago, sown with introduced pasture plants and since has been cultivated and sown with various crops and pastures. The sampling location was on Massey College farm near Palmerston North (Map ref. N.149, 099309) in two 1-acre plots of pure perennial ryegrass pasture maintained by Plant Chemistry Division, D.S.I.R. These plots were sown in 1956 and have been heavily fertilised with regular additions of ammonium sulphate and superphosphate.

(e) *Horotiu silt loam* was formed from rhyolite alluvium with mixed andesitic ash under manuka scrub and is classified as a yellow-brown loam. The area had been cleared and sown with introduced pasture plants about 90 years ago. The location of the sample sites was at the polo field, Claudelands Showgrounds, Hamilton (Map ref. N65, 798486), under a predominantly ryegrass-white clover pasture in the experimental area employed by the Ruakura Animal Research Station of the Department of Agriculture, for investigations on facial eczema disease. This area had been considerably modified as a result of development for a polo field and showground. Various dressings of soil and fertilisers had been made from time to time, and there had been much trampling by stock and repeated mowing.

Analysis and Determinations

Chemical analyses of soils were performed on samples taken from 0–6 in. by the methods described by Metson (1956) and results are shown in Table 1.

Moisture determinations were made in duplicate on samples collected at the 2–4 in. level at the beginning and end of each sampling period and the results given in Table 1 are the means of these determinations.

Temperature readings were taken from a thermometer thrust into the profile at the 3-in. level. Several readings were taken at the beginning and end of each sampling period and the mean results are given in Table 1.

Generalised descriptions of these soils are given by N.Z. Soil Bureau (1954).

Sampling Dates

Fungi in Kiwitea soil under forest and pasture, and Korokoro soil under pasture, were examined in October 1956; and in February and

TABLE 1. Chemical Analysis and Temperature and Moisture Relations of Some Soils Under Forest and Pasture

	Kiwitea silt loam		Gollans silt loam		Korokoro silt loam	Tokomaru silt loam	Horotiu sandy loam	
	Forest	Pasture	Forest	Pasture	Pasture	Pasture	Pasture	
pH, glass electrode	5.8	5.8			5.3	5.3	5.8	
Organic C %	11.7	8.0	4.9	4.6	5.3	3.2	4.0	
Total N %	0.96	0.65	0.46	0.41	0.34	0.28	0.34	
C/N	12	12	11	11	16	11	12	
Citric acid sol. P. mg % P.	9	11			3	2		
Cation Exch. Cap. me %	55.0	31.4	28.4	16.4	17.8	13.9	11.8	
Total Exch. Bases me %	44.4	16.3	12.1	5.2	6.4	6.0	9.6	
Base Saturation %	80	52	42	32	36	43	82	
Exch. Ca me %	36.8	13.6			3.2	3.9		
							Feb.	*Mar.*
Soil Temperature °C								
Spring	12	15			16	12		
Summer	16	21	17	22	23	21	26	21
Autumn	13	14			16	10		
Soil Moisture % D.W.								
Spring	74	56			20	39		
Summer	68	34	41	36	19	25	11	32
Autumn	72	50			28	33		
Soil Moisture g/100 cm^3 soil								
Spring	40	46			17	37		
Summer	36	23	39	29	15	26	15	33
Autumn	39	38			26	43		

April 1957. After the results had been obtained an examination was made in February 1958 of Gollans soil under forest and under pasture to determine if the same general results held in another site in another year. Tokomaru soil was sampled in October 1957, and in February and April 1958, to determine the microflora underneath a pure stand of perennial ryegrass and to provide comparison with Kiwitea and Korokoro soils at another site in another season. Horotiu soil was examined in the course of investigations of facial eczema disease (Thornton and Ross, 1960). Sampling in the latter was carried out under dry, summer conditions on 20–25 February (1957) and then on 12–16 March after 2½-in. rainfall on 9–10 March. This was the first of the autumn rains, and it was under such conditions that outbreaks of facial eczema had been known to occur in the past.

RESULTS AND DISCUSSION

Chemical and Physical Analyses of Soils

Table 1 shows that Kiwitea soil under pasture had lower organic carbon and total nitrogen contents than under forest although the C/N ratio was 12 in both cases. Only very slight changes were shown in the gleyed Gollans soil under these two types of vegetative cover. In both Kiwitea and Gollans soils the cation exchange capacities and total exchangeable bases were lower under pasture than under forest. This was probably due to the decreased return of bases to the surface under pasture. In a forest, bases in the deeper soil layers are tapped by tree roots and these bases are returned to the surface with the decomposition of the litter.

The Kiwitea and Gollans soils under forest were both cooler by 3–4°C and moister by 5–34% than under pasture as a result of the lower insolation and evaporation.

The various soils under pasture showed a range of exchange capacities from low in Horotiu to high in Kiwitea (Metson, 1956). The chemical analyses of Horotiu soil are atypical and show marked differences from other Soil Bureau analyses (N.Z. Soil Bureau, 1954). These changes no doubt are a result of the various practices employed in developing the area as a showground. The Korokoro soil showed a wider C/N ratio than the other soils, indicating a slower decomposition rate.

Summer temperatures in the pasture soils were 5–9°C higher than temperatures in either spring or autumn. On the Horotiu soil 2½ in. rain caused a drop in temperature. These pasture soils showed differences in moisture content expressed on a dry weight basis. Horotiu soil in February was the driest probably as a result of the sandy loam texture and the higher summer air temperatures in that area. The Korokoro soil is exposed to strong insolation and strong prevailing north-west winds. The Tokomaru and Gollans soils were wetter than these, the former because of impeded drainage and the latter because of a higher water table. The Kiwitea soil was surprisingly wet. This apparently high moisture content probably results from the content of light and porous rhyolitic pumice in these soils. As all samples for soil moisture determinations were taken with standard sized aluminium containers, of volume 16 cm^3, the moisture, in grammes, in 100 cm^3 of soil might give a clearer indication of the relative moisture states of the various pasture soils. Results are shown in Table 1.

Although some of the soils were examined in different years it is felt that the results show the relative moisture relationships well.

Mycelia in Soils

The total numbers of mycelia isolated from the different soils are given in Table 3, while the seasonal distribution of mycelia in some of the soils is given in Table 2. If the mean number of mycelia isolated per plate (Table 3) is taken as a measure of the mycelial status of the soil, particularly for those soils examined over three seasons, then the Kiwitea soil under forest had a lower mycelial status than the corresponding soil under pasture. Gollans soil, sampled only in summer, showed a similar but smaller difference. Chemical anayses of the soils indicate little that would account for this difference. However, there would certainly be a difference in the type and supply of organic additions to soils under forest and under pasture. No determinations of these were made in this study. The mycelial status of the well-drained Kiwitea and Korokoro soils under pasture appears to be very similar and was higher than that of Tokomaru soil under pasture, which was less well drained.

TABLE 2. INFLUENCE OF SEASON ON NUMBERS OF MYCELIA ISOLATED FROM FOREST AND PASTURE SOILS

	Spring	Summer	Autumn	Total
Kiwitea soil				
under forest	54	44	63	161
under pasture	78	111	96	285
Korokoro soil				
under pasture	97	103	76	276
Tokomaru soil				
under pasture	77	84	9	170

The seasonal isolations from Korokoro, Kiwitea and Tokomaru soils under pasture showed that summer did not have the favourable effect on the growth of mycelia that it did under tussock grassland, or oakwood and heath soil profiles (Thornton, 1956, 1958). The latter soils would have been cooler in spring and autumn than the pasture soils and were also of a lower fertility. The very low numbers of mycelial isolations from Tokomaru soil under pasture in autumn cannot fully be accounted for and can only partly be attributed to the low temperature and high moisture content at that time (Table 1). In Kiwitea soil under forest, isolations in summer appeared to be a little lower than in other seasons.

While these and other results suggest a seasonal influence on isolations of mycelia it was shown in Horotiu soil that 2½ in. of rain on a dry summer pasture was followed by lower soil temperatures, increased soil moisture levels and a 50% reduction in isolations of mycelia, when measured several days later. These results indicate that both season and immediate local weather influence fungal growth.

Occurrence of Species

Table 3 shows the distribution of those fungi more commonly isolated from the various soils. A complete list of the fungi isolated is given in Appendix A.

TABLE 3. OCCURENCE OF FUNGI FREQUENTLY ISOLATED BY SCREENED IMMERSION PLATES FROM SOME FOREST AND PASTURE SOILS

(Figures refer to the total numbers of isolations of mycelia for particular species from different soils.)

	FOREST		PASTURE					
FUNGI	Kiwitea Soil	Gollans Soil	Kiwitea Soil	Gollans Soil	Korokoro Soil	Tokomaru Soil	Horotiu Soil	
Season*	Sp.S.A.	S.	Sp.S.A.	S.	Sp.S.A.	Sp.S.A.	S. Feb.	Mar.
PHYCOMYCETES								
Pythium spp.	7	7	5	5	22	16		
Mucor hiemalis	1		16	6	12	12	1	
Absidia glanca					2		19	2
A. butleri						1	7	
Zygorhynchus moelleri			11			1		
Mortierella alpina	2	2	1		3			
M. spinosa	2		4		3			
FUNGI IMPERFECTI								
Cephalosporium acremonium				1		6	4	1
Trichoderma iride	7	1	29	5	66	4		3
Penicillium spp.	3	1			5		8	1
Gliocladium roseum		1	7		3	3	12	6
Humicola grisea	1		3		3			
Cladosporium herbarum	1	1			3	1	3	
Fusarium culmorum			53	16	12	27		
F. oxysporum			71	22	60	45	30	33
F. solani						4		
Cylindrocarpon olidum	8							
C. radicicola	50	21	20	1		5		
MYCELIA STERILIA								
Papulospora sp.	13							
Rhizoctonia solani			1	1			1	
Rhizoctonia sp.	19	2	11		19	2		1
Fungus S.B. 235	5	5	6		12			
Other non-sporing mycelia	29	7	39	1	35	28	12	3
Total numbers of mycelia isolated	161	48	285	60	276	170	108	52
Number of screened immersion plates employed	30	10	30	10	30	30	10	9
Mean number of mycelia isolated per screened immersion plate	5.3	4.8	9.5	6.0	9.2	5.6	10.8	5.6

* Sp. = Spring, S = Summer, A. = Autumn

It is clear from the table that Fungi Imperfecti were predominant in these soils and that under the two types of vegetation, forest and pasture, there were marked changes in fungal populations. In both Kiwitea and Gollans soils under forest, *Cylindrocarpon radicicola* was predominant,

yet in the corresponding soils under pasture, while this fungus was still present, Fusaria were predominant. Fusaria were not detected in the forest soils. Miller *et al.* (1957) found Fusaria to be rare in forest soils but very common in cultivated soils. Gordon (1956) also noted the absence of Fusaria in wooded soils.

Cylindrocarpon radicicola has been quite frequently isolated from soils by the screened plate method and appears to be a regular member of the active soil microflora. Harley and Waid (1955) isolated this fungus quite often from washed beech tree roots while Stenton (1958) found *Cylindrocarpon* to be by far the most abundant and constantly occurring root surface fungus on pea roots. Although *C. radicicola* was frequently isolated from tussock grassland soils it was not isolated from washed root portions of *Poa* and *Festuca* tussock plants (Thornton, 1958b). In Kiwitea soil under forest isolations of mycelia of the fungus were higher in spring and autumn than in summer. Results indicate that *C. radicicola* was isolated only from the wetter pasture soils.

In this study three species of *Fusarium* were isolated from pasture soils, *F. oxysporum* was isolated from all pasture soils, *F. culmorum* was present in four of the five soils and *F. solani* was isolated only from Tokomaru soil, under perennial ryegrass. Not only was *F. oxysporum* the most abundant *Fusarium* species but it was the predominant fungus in the pasture soils studied, constituting between 22 and 37% of total isolations in these soils. Gordon (1956) found this fungus to be the most prevalent species of *Fusarium* in a large number of Canadian soils and earlier had been found by him (Gordon, 1954) to be the most abundant *Fusarium* in soils of wheat field plots. Employing a hyphal isolation method Warcup (1957) isolated *F. oxysporum* and *F. avenaceum* from a wheat field soil, while Peterson (1958) found *F. oxysporum* to be common on wheat roots.

In the various soils under pasture the numbers of isolations of *Fusarium oxysporum* were highest in summer. During investigations of soil and leaf fungi possibly associated with facial eczema disease of sheep (Thornton and Ross, 1958) it was found that total mycelial isolations decreased markedly after 2½ in. rain had fallen on a dry summer pasture on Horotiu soil (Table 3). Isolations of *Fusarium oxysporum* however appeared to be unaffected by these conditions. *F. culmorum* was frequently isolated from pasture soils in this investigation. Waid (1957) found this fungus abundant on root surfaces and in the outer cortex of decomposing ryegrass roots, and in some cases in the inner cortex. This same fungus was reported to be a weak parasite of roots of many Gramineae (Griffiths and Siddiqi, 1958), there being antagonism with *Trichoderma viride* at certain temperature and moisture levels in soil.

Although *Fusarium* species are widely known for their pathogenic activities, particularly in the root rot complex in graminaceous plants, there was no indication from this study whether the strains isolated were pathogenic or not.

In Kiwitea soil under pasture, in addition to the occurrence of *Fusarium* mycelia in high numbers, *T. viride* and *Mucor hiemalis* both showed higher occurrence compared with the corresponding forest soil. Whereas isolations of *T. viride* were highest in summer, *Mucor hiemalis*

was relatively more common in spring, being practically absent in summer. *Pythium* spp. were often isolated and were present in four of the five soils under pasture and in both forest soils.

Non-sporing fungi, including *Rhizoctonia* sp., *Papulospora* sp. and Fungus S.B. 235, were quite frequently isolated. In Kiwitea soil under forest they contributed about 44% of isolations whereas in the pasture soils examined over the three growing seasons, i.e., Kiwitea, Korokoro and Tokomaru, they contributed between 20 and 33% of isolations. These figures are in contrast to those obtained in tussock grassland soils where non-sporing fungi formed approximately 60% of all isolations for the three soils studied (Thornton, 1958b).

Although *Papulospora* sp. was frequently isolated from Kiwitea soil under forest it was absent or not detected in that soil under pasture. It is interesting to note that another species of *Papulospora* which was commonly present in some tussock grassland soils declined in occurrence or was not detected in similar soils which had been sown with introduced pasture plants (Thornton, 1960b).

Non-sporing mycelia other than *Rhizoctonia*, *Papulospora* and Fungus S.B. 235 were quite frequently isolated and were mainly septate and non-septate white mycelia but included a number of dark forms.

Although fungi enter the screened plates by active mycelial growth it is not possible to determine the source from which mycelia originated, or how far these mycelia could spread from such sources. While there may be competition for entry and hence a limiting of the number of species isolated, possibly in favour of fast growing species of high competitive saprophytic ability, the present results are very similar to those obtained by Waid (1957) in England. He investigated the fungi within decomposing tissues of ryegrass roots by a method which involved washing and plating root portions. He found *Fusarium* spp.; non-sporing mycelia including *Rhizoctonia*; Zygomycetes including *Mucor hiemalis* and *Mortierella* spp.; *Penicillium* spp.; *Trichoderma viride*; *Gliocladium roseum* and *Cladosporium herbarum* to be the most commonly isolated fungi. These fungi were also among the very frequently isolated fungi in the present study of pasture soils. If in pasture soils fungi actively growing on and within decomposing grass roots are predominant, similarity between the fungal floras under pastures on different soils should be expected. Applying the concept of "soil fungal patterns" developed earlier (Thornton, 1956b, 1958b) it may readily be seen that the fungal patterns from most of the pasture soils were very similar (see Table 4). The different fungal pattern in Horotiu soil may be related to the treatment to which this area has been subjected.

Results from this and previous studies tend to suggest that the relative incidence of a species may be dependent on the higher-plant cover. Thus Fusaria while absent or not detected in broadleaf forest soils and in oakwood and heath soil profiles (Thornton, 1956b) were isolated often from tussock soils but not as frequently as from pasture soils. Stover (1956) and Jackson (1957) both found that spore dormancy in soil was broken in the presence of plant roots which suggested that root exudates had either overcome a fungistatic factor (Dodds and Hinson, 1953) or

TABLE 4. Soil Fungal Patterns of Species Contributing more than 5% of Isolations from Screened Immersion Plants, for Some Forest and Pasture Soils

(Numbers are percentage of total isolations from each soil.)

FOREST				PASTURE							
Kiwitea Soil		Gollans Soil		Kiwitea Soil		Gollans Soil		Korokoro Soil		Tokomaru Soil		Horotiu Soil (Feb. and Mar. results)	
Cylindrocarpon radicicola	31	*Cylindrocarpon radicicola*	41	*Fusarium oxysporum*	25	*Fusarium oxysporum*	37	*Trichoderma viride*	24	*Fusarium oxysporum*	27	*Fusarium oxysporum*	39
Non-sporing mycelia*	21	Non-sporing mycelia*	15	*Fusarium culmorum*	19	*Fusarium culmorum*	27	*Fusarium oxysporum*	22	Non-sporing mycelia*	17	*Absidia glauca glauca*	13
Rhizoctonia sp.	12	*Pythium* spp.	15	Non-sporing mycelia*	14	*Mucor hiemalis*	10	Non-sporing mycelia*	21	*Fusarium culmorum*	16	*Gliocladium roseum*	11
Papulospora sp.	8	Fungus S.B. 235	10	*Trichoderma* viride	10	*Trichoderma viride*	8	*Pythium* spp.	8	*Pythium* spp.	10	Non-sporing mycelia*	9
Cylindrocarpon olidum	5			*Cylindrocarpon radicicola*	7	*Pythium spp.*	8	*Rhizoctonia* sp.	7	*Mucor hiemalis*	7	*Penicillium* spp.	6
				Mucor hiemalis	6								

*Other than *Rhizoctonia* species, *Papulospora* sp. or Fungus S.B. 235

inadequate nutrition of the fungus. It is possible that the Gramineae by such action selectively encourage the development of Fusaria. As the density of grass roots is very high in pasture soils the fungal population must be considerably influenced by living, dying and dead roots and thus may possibly be regarded as being rhizospheric in the broad sense of the term (Thornton, 1958b). It is likely that similar effects may determine the relative occurrence of other species in other soils. Interactions between fungi and other soil organisms may also be of significance in determining the growth and survival of particular species. It is very interesting to find that *C. radicicola* and *Rhizoctonia* sp. were commonly isolated from forest soils, from tussock grassland soils (Thornton, 1958b) and from pasture soils. Possibly these species are universally distributed in New Zealand soils. Within a species or genus there will be many ecological races and it is possible that in different soil environments different "survival factors" will operate to enable the growth of particular races (Billings, 1957). In the soils of the present study several races would be included under particular species names. Ross (1960) sought to account for the changed incidence of some species by an investigation of the nutritional behaviour of strains of a number of species of fungi isolated from forest, pasture and tussock soils. He found no major difference in nutrition of different strains of a particular species from different grassland soils, or between strains which were predominant in some soils and occurred only infrequently in others. He found some differences between sporing species, e.g., Fusaria and *Cylindrocarpon radicicola*, and non-sporing forms, i.e., *Rhizoctonia* spp. and Fungus S.B. 235. Ross determined the optimal carbohydrate sources for each strain of each species. From these optimal sources the yields of dry mycelium of the non-sporing species were higher than those of the sporing species. However, the coefficient of variation in yields over the complete range of carbohydrates tested indicated that the non-sporing species were more selective than sporing species.

The similarity of the fungal patterns in the pasture soils and the similar nutrition of strains within species suggests that possibly, at least for fungi, a similar environment is being developed in the different soil types as the result of the establishment of the same higher-plant cover, the application of fertilisers, and by the same grazing animal -the sheep. The rate of fungal activity will possibly be influenced by the amount and rate of root exudate, dead root and leaves, and dung and urine additions.

ACKNOWLEDGMENTS

Chemical analyses of soils were carried out by the following: Misses F. A. D'Ath, N. V. Bailey, and Messrs M. F. Fitzpatrick, W. Owers, and L. C. Blakemore.

I wish to thank Dr W. L. Gordon, Dr H. C. Smith, and the Commonwealth Mycological Institute for examining and identifying a number of cultures.

REFERENCES

Billings, W. D. 1957: Physiological ecology. *Ann. Rev. plant Physiol. 8:* 375–92.

Chesters, C. G. C.; Thornton, R. H. 1956: A Comparison of Techniques for Isolating Soil Fungi. *Trans. Brit. mycol. Soc. 39:* 301–13.

Dobbs, C. G.; Hinson, W. H. 1953: A Widespread Fungistasis in Soils. *Nature, Lond. 172:* 197–9.

Gordon, W. L. 1954: The Occurrence of *Fusarium* Species in Canada. IV. Taxonomy and Prevalence of *Fusarium* Species in the Soil of Cereal Plots. *Canad. J. Bot. 32:* 622-9.

————1956: The Occurrence of *Fusarium* Species in Canada. V. Taxonomy and Geographic Distribution of *Fusarium* Species in Soil. *Ibid. 34:* 833–46.

Griffiths, E.; Siddiqi, M. A. 1958: Microbial Antagonism of *Fusarium culmorum. Nature, Lond. 182:* 956.

Jackson, R. M. 1957: Fungistasis as a Factor in the Rhizosphere Phenomenon. *Nature, Lond. 180:* 96–7.

Metson, A. J. 1956: Methods of Chemical Analysis for Soil Survey Samples. *N.Z. soil Bur. Bull. 12:* 208 pp.

Miller, J. H.; Giddens, J. E.; Foster, A. A. 1957: A Survey of the Fungi of Forest and Cultivated Soils of Georgia. *Mycologia, 49:* 779–808.

N.Z. Soil Bureau 1954: General Survey of the Soils of North Island, New Zealand. *N.Z. soil Bur. Bull,* n.s. *5:* 286 pp.

Peterson, E. A. 1958: Observations on Fungi Associated with Plant Roots. *Can. J. Microbiol. 4:* 257–65.

Ross, D. J. 1960: Physiological Studies of Some Common Fungi from Grassland Soils. *N.Z. J. Sci. 3:* 219–57.

Stenton, H. 1958: Colonisation of Roots of *Pisum sativum* L. by Fungi. *Trans. Brit. mycol. Soc. 41:* 78–80.

Stover, R. H. 1956: Studies on Fusarium Wilt of Bananas. I. The Behaviour of *F. oxysporum* f. *cubense* in different Soils. *Canad. J. Bot. 34:* 927–42.

Taylor, N. H.; Cox, J. E. 1956: The Soil Pattern of New Zealand. *Proc. N.Z. Inst. agric. Sci.:* 28–44.

Thornton, R. H. 1952: The Screened Immersion Plate. A method of Isolating Soil Microorganisms. *Research. 5:* 190–1.

————1956a: Phizoctonia in Natural Grassland Soils. *Nature, Lond. 177:* 230–1.

————1956b: Fungi Occurring in Mixed Oakwood and Heath Soil Profiles. *Trans Brit. mycol. Soc. 39:* 485–94.

—1958a: Biological Studies of Some Tussock Grassland Soils. I. Introduction, Soils and Vegetation. *N.Z. J. agric. Res. 1:* 913–21.

————1958b: Biological Studies of Some Tussock Grassland Soils. II. Fungi. *Ibid. 1:* 922–38.

————1960a: Biological Studies of Some Tussock Grassland Soils. XII. Soils and Vegetation of Two Cultivated Sites. *Ibid. 3:*

————1960b: Biological Studies of Some Tussock Grassland Soils. XIII. Fungi of Two Cultivated Soils. *Ibid. 3:*

————1960c: Growth of Fungi in Some Forest and Grassland Soils. *Growth of Soil Fungi.* Liverpool Univ. Press.

———; Ross, D. J. 1959: The Isolation and Cultivation of Some Fungi from Soils and Pastures Associated with Facial Eczema Disease of Sheep. *N.Z. J. agric. Res. 2:* 1002–16.

Waid, J. S. 1957: Distribution of Fungi Within the Decomposing Tissues of Ryegrass Roots. *Trans. Brit. mycol. Soc. 40:* 391–406.

Warcup, J. H. 1957: Studies on the Occurrence and Activity of Fungi in a Wheat-field Soil. *Trans. Brit. mycol. Soc. 40:* 237–62.

APPENDIX

?Pythium spp.
Mucor nr. *fragilis*. Bain
Mucor hiemalis. Wehmer
Mucor circinelloides. V. Tieghem
Mucor sp.
Absidia butleri. Lend
Absidia glauca. Hagem
Zygorhynchus moelleri. Vuill
Cunninghamella sp.
Mortierella alpina. Peyr
M. elongata. Linm
M. gracilis. Linm
M. hygrophila. Linm
M. minutissima. V. Tieghem
M. polycephala. Coemans
M. spinosa. Linm
?Coniodictyium sp.
Cephalosporium acremonium Cda
C. nr. *curtipes*. Sacc.
?Fusidium sp.
Trichoderma viride. Pers. ex Fr.
4 *Penicillium* spp.
Gliocladium roseum. Bain.
Paecilomyces sp.
Verticillium sp.
Fusariella sp.
Sporotrichum sp.
Humicola grisea. Traen
Hormodendron hordei. Bruhne
Cladosporium herbarum. Link
Torula sp.
Helminthosporium sp.
Trichocladium opacum. (Cda) Hughes
Stemphylium sp.
Alternaria sp.
Fusarium culmorum (W. G. Smith) Sacc.
F. oxysporum. Schlecht. emend Snyder & Hansen.
F. Solani. (Mart.) App & Wr. emend Snyder & Hansen.
Cylindrocarpon radicicola. Wr.
C. olidum. Wr.
Rhizoctonia solani. Kuhn
Rhizoconia sp.
Papulospora sp.
Fungus S.B. 235
Other non-sporing mycelia.

PHOTOSENSITIVITY DISEASES IN NEW ZEALAND

XXII. STUDIES IN THE EFFECT ON THE RABBIT OF INJECTION OF EXTRACTS CONTAINING FACIAL ECZEMA TOXIN

By N. A. Worker and D. C. Dodd, Ruakura Animal Research Station, Department of Agriculture, Hamilton

(*Received for publication, 1 June 1960*)

Summary

An investigation has been undertaken into the effects of toxic extracts of *Sporidesmium bakeri,* Syd.* administered parenterally in the rabbit, in an attempt to obtain information on the behaviour of the facial eczema toxin within the animal.

Lesions characteristic of facial eczema liver damage were found after injection of toxic extracts into the portal system. Macroscopic damage was first observed 48–96 hours after injection. The earliest evidence of histological change was noted 3–6 hours after injection.

Liver damage was consistently noted after injection of extracts into the duodenum and gall-bladder, rarely after injection into the systemic circulation via the jugular or ear vein, but never after injection into the caecum.

The significance of these findings is discussed in relation to the occurrence of liver damage in animals in the field. It is concluded that the toxin is absorbed through the wall of the intestine and transported by way of the portal circulation to the liver where it is removed instantaneously by the liver cells.

The possible involvement of the gall-bladder in the pathogenesis of liver damage is discussed.

Introduction

An acute cholangitis is generally recognised as the essential feature of facial-eczema liver damage in the sheep, (Cunningham *et al.* 1942; McFarlane *et al.* 1959). It has recently been demonstrated that this condition is caused by a toxic principle elaborated by the fungus *Sporidesmium bakeri,* Syd., a common saprophytic organism inhabiting pastures in many areas in this country (Thornton and Percival, 1959; Percival, 1959). Guinea pigs and rabbits have also been shown to be susceptible to the disease as liver damage of a generally similar nature has been produced by feeding toxic grass and cultures of the fungus (Evans *et al.,* 1957; Perrin, 1957; Clare, 1959) and ether extracts of toxic grass and fungus (Perrin, 1957; Clare, 1959).

Comparatively little is known concerning the behaviour of the toxin in the animal body. On the basis of indirect evidence, obtained in the course of studies into the histopathology of the disease, it is generally assumed that the toxin is passed to the liver via the portal circulation and excreted into the biliary system where an acute inflammatory reaction is produced causing damage to the epithelial

* Since this paper went to press the toxic fungus has been reclassified as *Pithomyces chartarum* (Berk. & Curt.) M. B. Ellis.

lining of the ducts (McFarlane *et al.*, 1959). There is at present, however, no direct experimental evidence to support these assumptions, nor is there any indication of the time relationships involved in the development of early liver damage or of the ultimate fate of the toxin within the animal.

Attempts have been made recently at this Station to study certain of these problems. The present communication describes the results of a series of investigations into the utilisation of toxic extracts of fungus administered by various routes other than the oral. The work was undertaken in the rabbit since this species has been shown recently to be highly susceptible to facial-eczema liver damage (Clare, 1959) and is, in addition, particularly suitable for studies involving experimental surgery. The results described provide information not only on the relative effectiveness of the toxin when administered by different routes, but also evidence relating to the absorption and transport of the toxin and its rate of uptake from the blood into the liver.

Experimental Procedure

Rabbits

New Zealand White Rabbits from the Station colony were used in all experiments. The animals were fed *ad lib* on pellets of the following percentage composition: ground wheat, 36.0; ground barley, 10.0; ground oats, 4.0; ground maize, 6.0; dehydrated lucerne meal, 10.0; meat meal, 21.0; dried blood, 1.0; liver meal, 0.5; skim milk powder, 11.0; salt, 0.5. This diet was also supplemented with fresh grass or cabbage daily.

Fungal cultures

Cultures of *Sporidesmium bakeri*, Syd. were grown on potato-carrot medium (see Thornton and Ross, 1959) under the supervision of Mr. W. E. Crawley, of the Veterinary Diagnostic Section. The cultures were grown for 5–7 days and then ether extracted in bulk and subdivided at the extract stage for use by different workers.

Preparation of Extracts

Ether extracts were prepared from the whole fungal culture (mycelial felt + culture medium) using the method described by White (1959). The usual dry-matter yield of such extracts, after removal of "beaker test" material, was about 100 mg (range approximately 70–130 mg) per litre of culture.

Preparation of Aqueous Dispersions of Extract

For injection purposes extracts were dispersed in 10% aqueous Tween 40 (Atlas Powder Co.) using a Potter-Elvehjem homogenizer. As a routine, the extract was usually made up at a concentration of 100 mg/ml Tween solution.

Injection of Dispersions

Injections into the systemic circulation were made by ear vein or jugular vein. In the latter case a small neck incision was made under a general anaesthetic. Injections into the portal circulation were made into one of the small collecting veins of the mesentery after laparotomy

under general anaesthesia. Intracaecal and intraduodenal injections were given under general anaesthesia after laparotomy, as were also injections into the gall-bladder. Intraperitoneal injections were made without use of an anaesthetic.

Anaesthetic

Veterinary "nembutal" pentobarbital sodium (Abbott Laboratories Ltd) was used when general anaesthesia was required. The stock solution was diluted with an equal volume of sterile saline prior to use, and administered by ear vein at the rate of 1.65 ml (dilute solution) per kg body weight.

Post Mortem Examination and Histology

Morbid anatomy and histological examinations were carried out on livers taken post-mortem. The livers were graded both macroscopically and histologically into the following arbitrary gradings: 1, Normal; 2, Mild; 3, Moderate; 4, Severe; 5, Very Severe. The significance of these gradings has been discussed elsewhere by Dodd (1959).

Results

Effect of Injection of Toxic Extract into the Portal Circulation

The results of a preliminary series of experiments, in which toxic extracts were injected at different dosage levels into the portal circulation, are summarised in Table 1. The animals were killed 8 days after dosing as this period had previously been shown in feeding trials with rabbits to be sufficient to allow development of liver damage (Clare, 1959).

The results of these initial experiments clearly indicated that facial-eczema liver lesions, indistinguishable macroscopically and histologically from those obtained on feeding toxic grass or fungal culture, could be produced by injection of toxic extracts into the portal circulation, the extent and severity of the damage being roughly proportonal to dosage level. A dose of 20 mg ether extract per kg body weight was the lowest level at which damage could be detected histologically. Macroscopic lesions were first discernible in animals dosed at 40 mg extract per kg body weight. The most severe lesions were produced with a dose of 160 mg/kg body weight, the highest level injected. Two out of four animals in this group barely managed to survive for the duration of the experiment.

A most consistent histological feature of many of the livers examined was the presence of an acute phlebitis, usually in the larger portal branches. The periphery of these lesions was, in many cases, made up of a dense mass of inflammatory cells (mainly eosinophils) and cell debris extending in some cases into the surrounding liver parenchyma. Normal red cells were seen to be present usually in the centre of such lesions, but in others complete occlusion of the vein was evident due to thrombus formation.

Livers from animals injected with 1 ml or 2 ml of aqueous Tween 40 alone were macroscopically normal at slaughter and on routine histological examination showed no apparent abnormalities.

Effect of Interval Between Dosing and Slaughter on Development of Liver Lesions

In an attempt to study the development of liver damage after injection, animals were injected intraportally with a standard dose of toxic extract and slaughtered at intervals of 3, 6, 12, 24, 48 and 96 hours. The results are summarised in Table 2. Results of a comparable group killed at 8 days are presented in Table 1 (group 3). To ensure an adequate dose of toxin, toxic extract at a level of 80 mg/kg body weight was administered. Young rabbits weighing 400–600 g were used in the experiment, four such animals being allotted to each treatment group.

On post mortem examination all animals in Group 6, and two out of the four animals in Group 5, showed macroscopic lesions consistent with facial-eczema liver damage. None of the animals in the other groups showed any apparent macroscopic abnormalities.

On histological examination every animal in Group 5 and 6 showed characteristic facial-eczema liver damage, similar to that described by Dodd (1960). In Groups 3 and 4, two animals, and one animal respectively, showed no significant microscopic changes. The other animals in each group, however, exhibited mild to moderate periductal oedema and fibrosis of the larger bile ducts with generalised portal thrombophlebitis and, in occasional sections, a marked central zone liver cell degeneration. All animals in Groups 1 and 2 showed a fairly generalised portal thrombophlebitis and, in some, a central zone liver cell degeneration was apparent. Of the animals in Group 1, three exhibited a mild periductal oedema and fibrosis of the larger ducts, and of the animals in Group 2, two showed a mild to moderate periductal oedema and fibrosis. Examples of the damage seen at post-mortem and on histological examination are shown in Figs. 1–8. There was no evidence of any abnormalities in the livers of the twelve control animals injected with aqueous Tween 40 alone.

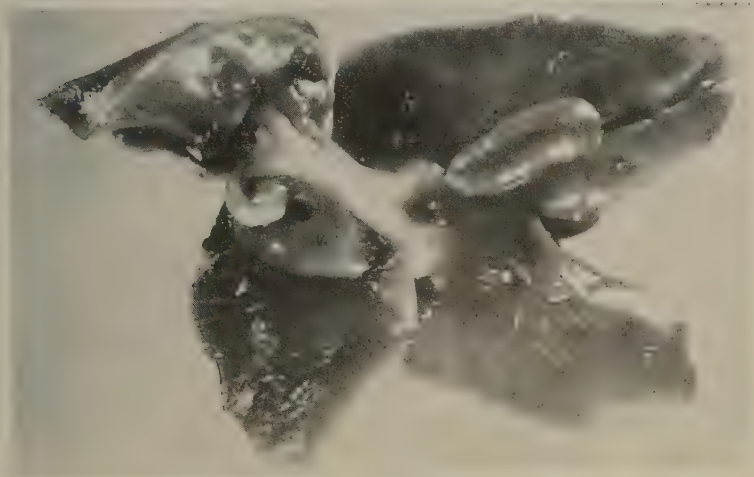

Fig. 1.—Moderate facial eczema liver damage occuring 96 hours after intraportal injection of toxic extracts (R31, Table 2).

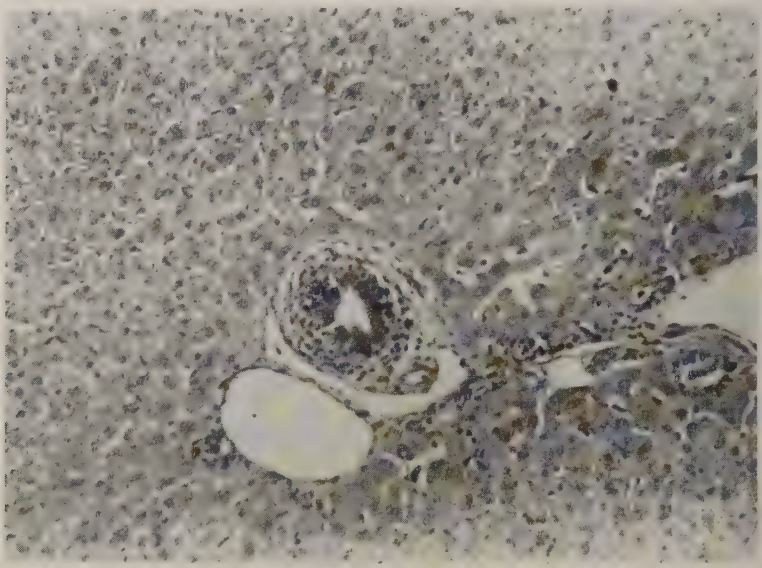

Fig. 2.—Mild periductal oedema and fibrosis of larger bile duct seen 3 hours after injection of extract (R1, Table 2) × 133. H. and E.

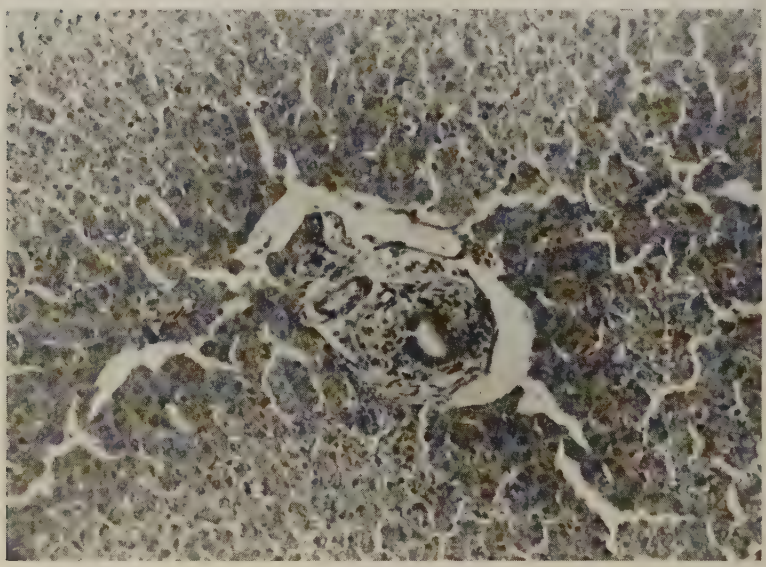

Fig. 3.—Mild periductal oedema and fibrosis of a larger bile duct seen 6 hours after injection of extract (R7, Table 2) × 133. H. and E.

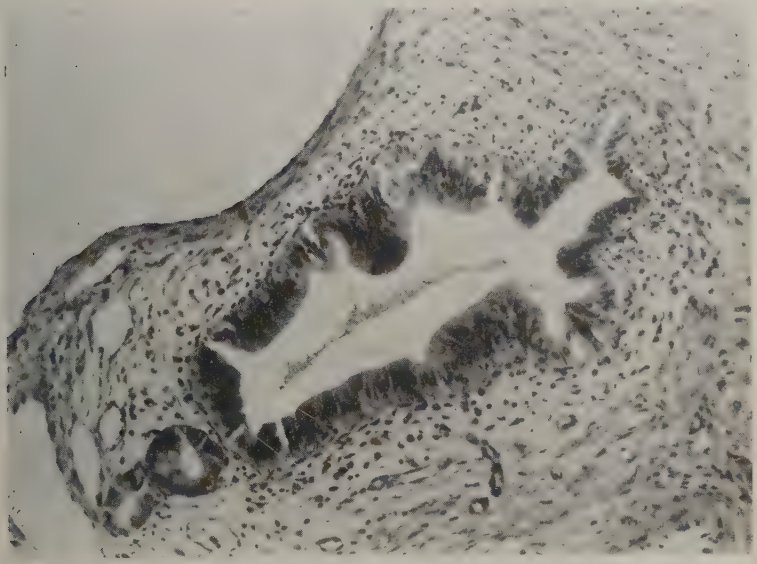

Fig. 4.—Moderate periductal oedema and fibrosis of a large bile duct seen 24 hours after injection of extract (R19, Table 2) × 133. H. and E.

Fig. 5.—Marked degenerative changes of the gall-bladder 24 hours after intraportal injection of extract (R20, Table 2) × 133. H. and E.

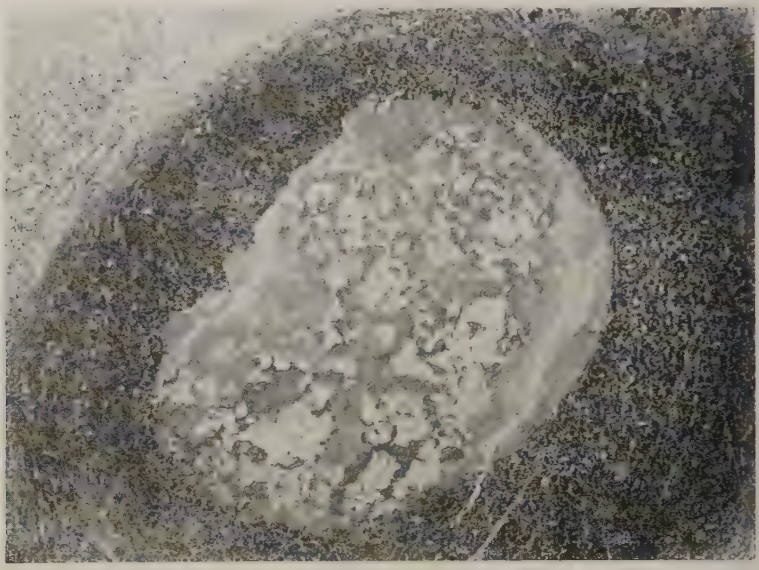

Fig. 6.—Severe phlebitis in a portal radicle 48 hours after injection of extract (R27, Table 2) × 53. H. and E.

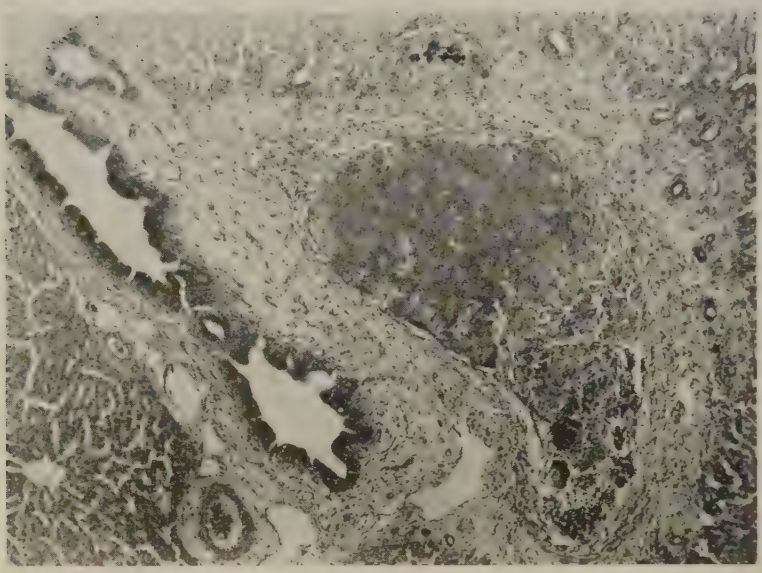

Fig. 7.—Marked periductal inflammatory fibrosis and thrombosis of portal vein. (R31, Table 2) × 53. H. and E.

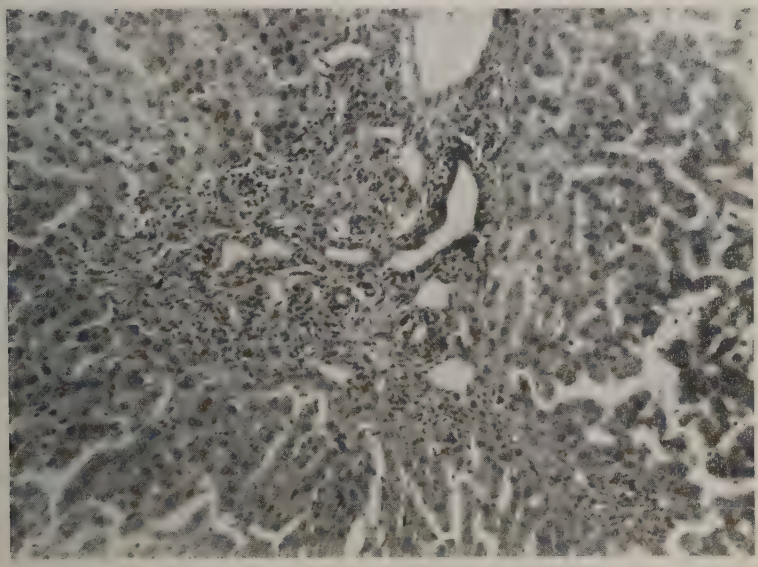

Fig. 8.—Hyperplasia of bile ductules in portal radicle near periphery of lobe and proximal to an occlusion (R31, Table 2) × 133. H. and E.

Effect of Route of Administration

In order to obtain information on the relative effectiveness of toxic extracts when administered by different routes, several experiments were carried out in which comparable doses of toxic extract were injected into: (1) the portal circulation; (2) the systemic circulation; (3) the duodenum, proximal to the sphincter of Oddi; (4) the caecum, in the region of the ileocaecal valve; and (5) the gall-bladder, in the vicinity of the ampulla. For this purpose extract was dispersed in 20% aqueous Tween 40 at a concentration of 200 mg/ml in order to reduce to a minimum the total volume of material to be injected into the gall-bladder, the capacity of which in animals of the size used in the present experiments was about 0.4–0.5 ml. At no time was sufficient extract available to perform large scale experiments in which extracts of the same batch could be used for simultaneous tests of the different routes of administration. Each experiment was controlled, however, by the positive result obtained in each case by the intraportal administration of the particular extract being used. On the basis of the results obtained in the previous studies, a standard dose of extract of 80 mg/kg was decided upon and the animals were killed at periods varying from 4 to 8 days after dosing. Animals ranging in weight from 1000 to 1500 g were used.

The combined results of the several experiments are reported in Table 3. After injection of extract into the duodenum or the portal circulation, macroscopic lesions of varying severity consistent with facial-eczema liver damage were found. The majority of animals in each group showed mild or moderate liver damage, the extent and severity of the

lesions being confirmed histologically. On injection of comparable amounts of extract into the ear vein or jugular vein, on the other hand, no evidence of any gross liver damage was found and all livers in the group, with the exception of two, were histologically normal. The two positive livers were graded as mild on histological examination. Injection of extracts into the caecum likewise produced negative results, the livers from animals in this group being both macroscopically and histologically normal at slaughter. In contrast to these findings, injection into the gall-bladder produced, in all animals, lesions of a most severe form. This treatment resulted inevitably in severe necrosis and complete functional obliteration of the gall-bladder, with very severe generalised oedema and fibrosis of the extra- and intra-hepatic biliary systems, together with gross enlargement of the liver and marked discolouration. A very significant feature of the results was the completely generalised effect of the treatment noted throughout all lobes of the liver. Fifteen of the twenty-six animals so treated exhibited clinical icterus, a condition which was not noted in any of the other experimental groups.

Discussion

Several workers have reported on the histopathology of facial eczema (for review see Dodd, 1959) and the essential features of the disease have, for the most part, been well defined. Little attempt has been made up to the present time, on the other hand, to elucidate the pathogenesis of this condition. This has been due in part to a lack of suitable toxic material with which to undertake such work and of effective techniques for studying the behaviour of the toxin *in vivo*. The discovery of the fungal origin of the toxin by Thornton and Percival (1959) however, which allowed large-scale culture of the toxic fungus, together with the systematic chemical work of White (1958, 1959) aimed at chemical isolation of the toxin, have recently combined to provide concentrated toxic material of a type suitable for physiological studies.

It was obvious at the outset of the present work that the success of studies into the behaviour of the toxin was dependent very largely on the development of techniques for administering the toxin by routes other than the oral. The present experiments were undertaken, therefore, in an attempt to establish these techniques and to obtain preliminary information on the overall usefulness and practicability of the parenteral approach.

The results obtained in the initial experiments of the series (Table 1) showed that lesions, indistinguishable from those occurring in animals fed toxic grass or fungus, could be produced by injection of toxic extract into the portal circulation. The results of this particular experiment, in addition, provided necessary information on suitable dosage levels of toxin for use subsequently and served to confirm the general suitability of the rabbit as the species of choice for such work.

In those studies in which the development of liver damage was followed at intervals after intraportal injection of toxic extract (Table 2), a definite sequence of changes was apparent. Typical macroscopic lesions were first observed 48 hours after dosing and at 96 hours advanced

TABLE 1. Intraportal Injection of Different Levels of Toxic Extract Dispersed in 10% Aqueous Tween 40. (Animals Killed 8 Days After Injection)

	Groups					
	1	2	3	4	5	6
Mean Wt. at Injection (g)	1170	1210	1260	1240	1120	1140
Dose of extract (mg/kg body wt.)	20	40	80	160	1 ml Tween alone	2 ml Tween alone
Macroscopic Liver Grading						
Normal	4	2	0	0	4	4
Mild	0	1	1	0	0	0
Moderate	0	1	2	1	0	0
Severe	0	0	1	3	0	0
Histological Liver Grading						
Normal	2	1	0	0	4	4
Mild	2	1	0	0	0	0
Moderate	0	1	1	0	0	0
Severe	0	1	3	4	0	0

damage was apparent, indicating the rapidity with whcih gross changes become evident following a single toxic dose. Owing to the limited supplies of toxic extract which were available at the time, the number of animals which could be used in each treatment group was small and for this reason it was not possible to obtain an accurate and detailed picture of the progressive histological changes which occurred following injection. The results showed, however, that an inflammatory reaction in bile duct epithelial tissues occurs rapidly after injection and in some cases may appear within 3–6 hours (see Figs. 2 and 3) which is an observation of considerable interest. If, as suggested by McFarlane *et al.* (1959), bile duct damage is caused by the presence in bile of the toxin or of an active metabolite, then it would appear that transfer of the active material from the bloodstream through the liver and into the bile is a relatively rapid process.

The failure to produce lesions except in two livers following injection of extract by the jugular or ear vein is difficult to explain. Several factors can be suggested, such as dilution and inactivation, or instability of the toxin in this rather crude extract form. In a more recent investigation with two rabbits, gross liver damage was not produced in eight days even with an intravenous dose of the same type of extract of 400 mg/kg bodyweight. The clarification of this point must await the results of further work which is now being done with purer extracts of the toxin.

In the experiments reported, it is suggested that if the toxin was, in fact, inactivated, the destruction would probably have occurred in the pulmonary capillary bed. If the whole of the toxic material injected was still present in an active form in arterial blood leaving the heart

TABLE 2. DEVELOPMENT OF LIVER LESIONS AFTER INTRAPORTAL INJECTION OF TOXIC EXTRACT IN TWEEN (Dose = 80 mg/kg body weight)

Group	Animal Number	Time in hours Between Inj. & Slaughter	Macroscopic grading of liver damage	Abbreviated Histological Report of liver damage
1	1	3	Normal	Mild—portal phlebitis and periductal oedema (see Fig. 2)
	2	,,	,,	,,
	3	,,	,,	,,
	4	,,	,,	Mild—phlebitis only
	5*	,,	,,	Normal
	6*	,,	,,	,,
2	7	6	Normal	Moderate—phlebitis and periductal oedema & fibrosis (see Fig. 3)
	8	,,	,,	,,
	9	,,	,,	Marked general phlebitis
	10	,,	,,	,,
	11*	,,	,,	Normal
	12*	,,	,,	,,
3	13	12	Normal	General phlebitis and moderate periductal oedema and fibrosis in portal areas
	14	,,	,,	,,
	15	,,	,,	Normal
	16	,,	,,	,,
	17*	,,	,,	,,
	18*	,,	,,	,,
4	19	24	Normal	Marked periductal oedema & fibrosis with many eosinophils—phlebitis (see Fig. 4)
	20	,,	,,	,, also complete loss of epithelial cells of gall bladder mucosa (see Fig. 5)
	21	,,	,,	As for 19
	22	,,	,,	Normal
	23*	,,	,,	,,
	24*	,,	,,	,,
5	25	48	Moderate	Moderate generalised damage as above—focal areas of necrosis of biliary epithelium
	26	,,	Normal	Mild generalised damage in portal radicles
	27	,,	Mild	Moderate generalised damage in portal radicles (see Fig. 6)
	28	,,	Normal	Moderate damage to medium sized and larger ducts
	29*	,,	,,	Normal
	30*	,,	,,	,,
6	31	96	Moderate	Severe generalised damage in portal areas—marked degeneration of biliary epithelium—focal coagulation necrosis of liver cells (see Figs. 1, 7 & 8)
	32	,,	Mild	,,
	33	,,	Severe	,, but more marked
	34	,,	,,	,,
	35*	,,	Normal	Normal
	36*	,,	,,	,,

* Injected with Tween alone

TABLE 3. Grading of Liver Damage, Based on Macroscopic and Histological Examination After Administration of Toxic Extract in Tween by Different Routes (Dose 80 mg/kg body weight)

Route of Administration	Grading				
	Normal	Mild	Moderate	Severe	Very Severe
1. Portal circulation	1	9	17	6	2
2. Systemic circulation	33	2	0	0	0
3. Duodenum	2	11	15	7	0
4. Caecum	20	0	0	0	0
5. Gallbladder	0	0	0	0	26

after traversing the pulmonary circulation, an amount sufficient to induce liver damage would have been expected to have reached the liver directly via the hepatic artery and portal vein which together receive upwards of 20% of the total arterial supply (Lichtman, 1942). These observations also suggest that, in animals in the field, lymphatic uptake of toxin is probably of little significance insofar as the development of liver damage is concerned, and considered along with the apparent inability of the toxin to produce any response when injected into the caecum, provide support for the hypothesis that absorption through the intestinal wall and transport via the portal circulation is the main pathway by which the toxin enters the liver. The close similarity in the effectiveness of the toxin whether injected into the duodenum or portal system indicates also that transfer of the toxin through the wall of the intestine into the blood-stream must be a relatively efficient process. Insofar as the rate of uptake of toxin by the liver is concerned, the fact that the toxin may be inactivated on entering the general circulation suggests that liver damage is produced by toxin which is removed from the portal blood the first time it passes through the liver sinusoids, and that under these circumstances, therefore, removal of this toxin by the liver cells must be immediate.

A satisfactory explanation of the results obtained after injection of toxic extract directly into the gall-bladder is not easy in terms of the usual concepts of liver physiology and function. In particular, the extreme severity of the lesions and their generalised distribution throughout the organ are puzzling.

Movement of the dose into the intestine followed by its subsequent re-absorption into the portal circulation is clearly not a sufficient explanation for these effects, since injection of equivalent amount of toxin into the duodenum or portal system produced damage of very much less severity. Similarly, absorption of the dose by way of the venous drainage of the gall-bladder would appear unlikely to be a factor of any significance, since cystic venous blood is discharged into the portal circulation. One possible explanation is that there is a retrograde movement of toxin from the gall-bladder along the biliary tree, although such a movement is difficult to visualise against a bile flow which, in the rabbit, is about 8 ml/kg/hour (Worker, unpublished results). Such a movement would, however, provide an adequate explanation for the extreme severity of the lesions and for the uniform distribution of the damage

throughout the organ. This hypothesis requires further consideration, not only in view of its general interest, but also because of its possible practical significance. If after concentration and storage in the gall-bladder, materials re-enter and move within the biliary tree, it suggests that in animals ingesting the toxin under field conditions, the function of the gall-bladder and biliary apparatus may possibly play an important role in the pathogenesis of liver damage. Further work is required to examine this possibility in more detail.

Acknowledgments

The authors wish to acknowledge the collaboration of the following persons: Mr W. E. Crawley for growing the fungal cultures; Messrs A. Fyvie and H. de Langen for ether extraction of the cultures; Misses Gillian Hughes and Ruth Henson for technical assistance with the surgery and care of the animals and Mr. J. Kerr for the preparation and staining of sections for histological examinations.

References

Clare, N. T. 1959: *N.Z. J. Agric. Res. 2:* 1249–56.

Cunningham, I. J.; Hopkirk, C. S. M.; Filmer, J. F. 1942: *N.Z. J. Sci. Tech. A24:* 185.

Dodd, D. C. 1959: *Proc. N.Z. Soc. Anim. Prod. A24:* 185.

———— 1960: *N.Z. J. Agric. Res. 3:* 491–7.

Evans, J. V.; McFarlane, D.; Reid, C. S. W.; Perrin, D. D. 1957: *N.Z. J. Sci. Tech. A38:* 491–6.

Lichtman, S. S. 1942: "Diseases of the Liver Gallbladder and Bile Ducts"; Lea and Febiger, Philadelphia.

McFarlane, D.; Evans, J. V.; Reid, C. S. W. 1959: *N.Z. J. Agric. Res. 2:* 194–200.

Percival, J. C. 1959: *Ibid. 2:* 1041–56.

Perrin, D. D. 1957: *N.Z. J. Sci. Tech. A38:* 669–79.

Thornton, R. H.; Percival, J. C. 1959: *Nature,* Lond. *183:* 63.

Thornton, R. H.; Ross, D. J. 1959: *N.Z. J. Agric. Res. 2:* 1002–16.

White, E. P. 1958: *Ibid. 1:* 433–46.

———— *Proc. N.Z. Soc. Anim. Prod. 19:* 64–8.

BREEDING SYSTEMS IN NEW ZEALAND GRASSES

II. GYNODIOECY IN *Arundo richardii* Endl.

By H. E. Connor and E. D. Penny,* Botany Division, Department of Scientific and Industrial Research, Christchurch

(*Received for publication, 7 June 1960*)

Summary

Dimorphism in flowers of *Arundo richardii* is described, and gynodioecy is established as the breeding system.

Introduction

Attention was drawn to dimorphism in the flowers of *Arundo* by Mr V. D. Zotov of the Botany Division, who recognised the presence of a form of dioecy in the species. Investigations during late December 1959 through to mid-February 1960 have lead to the conclusion that *Arundo richardii* is gynodioecious.

Gynodioecy is an outcrossing breeding system of inter-breeding hermaphrodites and females, even though the hermaphrodites may be self-pollinated at times. Lewis and Crowe (1956) point out that gynodioecy differs from incompatibility and dioecy in that in gynodioecy the two mating types contribute unequal numbers of nuclear genes to the next generation. Male sterility, a condition associated with gynodioecy, has been reported in grasses, and these are listed in van der Kley (1954), but in these cases male sterility has not become permanently established in the species. True gynodioecy does not appear to have been reported in the Gramineae though it is well known in some other families.

Arundo richardii, toetoe, the only species examined, commonly forms stands on river flood plains, sides of streams, and in seepage areas, and where liable to flooding and silting there is often a marked vegetative extension of the clumps.

Floral Dimorphism

A sample of 84 plants growing on the flood plain of the Mason River, North Canterbury, was collected on 28 December 1959. Fifty plants (59.5%) were hermaphrodite, and 34 (40.5%) were female. At this time the plants were at anthesis and no seed had been set. Floral dimorphism was very clear, the female plants having long prominent stigmas and short white non-dehiscing empty anthers while the hermaphrodites had short stigmas and yellow well-filled anthers. This area was resampled on 2 February 1960 together with a further population from the opposite side of the Mason River. From the first site all 123 plants sampled had set seed, and from the adjacent population 121 plants were sampled and had all set seed. In these two collections there were six plants (three hermaphrodites and three females) still at anthesis. Stigmas and anthers

* Present address—Botany Department, University of Canterbury, Christchurch.

were measured from these six plants and the results are summarised in Table 1. Anther lengths from hermaphrodite and female plants were to a highly significant degree different as were stigma lengths. Anther length in hermaphrodite plants ranged from 2.6 to 4.2 mm, and there was no overlap with anther length from female plants which ranged from 1.7 to 2.3 mm. In stigma length there was a slight overlap in the ranges, hermaphrodite—1.2–2.6 mm, female—1.9–4.0 mm. The distribution in 0.5 mm classes for both stigma and anther length is shown in Fig. 1.

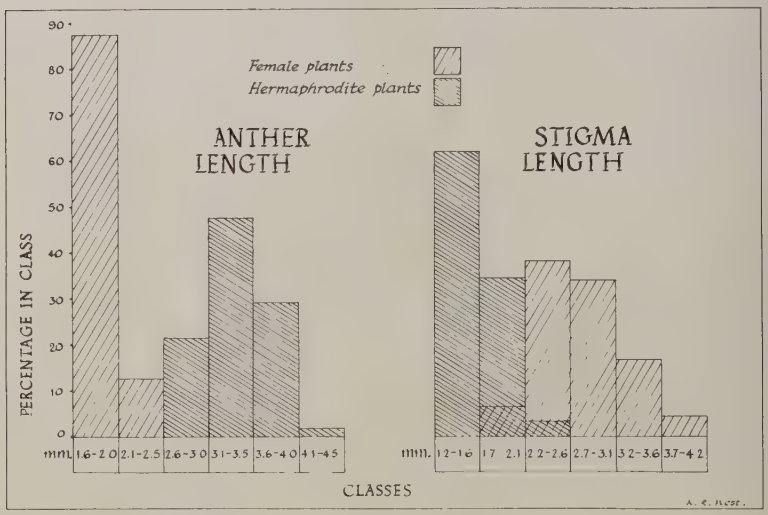

Fig. 1.—Distribution of stigma and anther lengths in *Arando richardii*.

Table 1. Anther and Stigma Lengths in Six Plants of *Arundo richardii* From Mason River

	Mean Anther Length (mm)	Standard Deviation	n	Mean Stigma Length (mm)	Standard Deviation	n
Hermaphrodite	3.32 ± 0.04	0.34	65	1.57 ± 0.06	0.33	29
Female	1.95 ± 0.02	0.13	32	2.84 ± 0.04	0.27	47

Breeding System

It may be concluded from the fact that good seed had set in both the female and hermaphrodite plants that gynodioecy is the breeding system in this species. There is no evidence to suggest either apomixis or parthenocarpy, this latter being ruled out by the normal formation of all seeds examined.

Four other collections of *Arundo richardii* were also made, three in North Canterbury and one in North Otago. These four samples had set

seed at the time of collection and it was therefore not possible to determine the ratio of hermaphrodites to females. The number of seed-bearing plants varied (see Table 2) and only once approached the 100% level of the Mason River collections.

TABLE 2. SEED-BEARING PLANTS IN FOUR COLLECTIONS OF *A. richardii*

Locality	Plants setting seed	Plants not setting seed	Total
Leslie Hills Bridge, Waiau River, 29.12.59	92 (92%)	8 (8%)	100
Waiau River, 1 mile east of Bridge, 31.12.59	65 (75.6%)	20 (23.5%)	85
Waiau River Bridge, 2.1.60	101 (80.8%)	24 (19.2%)	125
Wairepo Stream, near Lake Ohau, 26.1.60	86 (78.2%)	24 (21.8%)	110

It is impossible to say whether or not the plants lacking seed were in fact sterile. It may be pointed out that the two Waiau Bridge collections and that from Wairepo Stream are very similar in the proportion of seed-bearing plants found, and this may indicate some common level of sterility by comparison with the full fertility seen in the Mason River and Leslie Hills Bridge collections.

Plants are being grown for investigation of the genetic system controlling gynodioecy in this species, and to determine whether apomixis occurs. This project will inevitably be prolonged as the time from seed to flowering is expected to be three or four years.

REFERENCES

Kley, van der K. F. 1954: Male Sterility and its Importance in Breeding Heterosis Varieties. *Euphytica 3:* 117–24.

Lewis, D.; Crowe, L. K. 1956: The Genetics and Evolution of Gynodioecy. *Evolution 10:* 115–25.

BREEDING SYSTEMS IN NEW ZEALAND GRASSES

III. FESTUCEAE, AVENEAE AND AGROSTIDEAE

By H. E. Connor, Botany Division, Department of Scientific and Industrial Research, Christchurch

(*Received for publication, 7 June 1960*)

Summary

Studies on the breeding systems of five species of indigenous grasses *Festuca novae-zelandiae*, *F. matthewsii*, *F. multinodis*, *Koeleria kurtzii*, and *Deyeuxia billardieri* are reported on, together with some notes on seed setting in *Danthonia rigida*.

Introduction

In previous papers (Connor and Cook, 1952, 1955; Connor, 1954, 1957; Connor and Penny, 1960) the breeding systems of 17 indigenous species of grasses were described. Among these were 11 species showing self-fertility and no inbreeding depression in S_1 progenies, two were somewhat self-fertile but with degenerate progenies, and three were self-sterile. In this paper further results from trials on *Festuca novae-zelandiae* are reported which support the conclusions previously reached, while the breeding systems of four species are discussed for the first time, and a few notes on some other species are included. With the addition of the species dealt with here the breeding systems of 21 indigenous grasses will have now been reported.

Materials and Methods

Plants from all species have been grown in the Botany Division experimental gardens either from seed collected in the wild, or as wildings transferred to the gardens. The methods for self-pollination have been described previously (Connor and Cook, 1952). In most cases the determination of the effects of selfing have been made from measurements of plant height and from counting the number of culms produced. When inbreeding depression is present, it is readily seen in these quantitative characters.

Results

Tribe Festuceae

Festuca novae-zelandiae (Hack.) Cockayne. Previous results from a small trial between S_1 and open-pollinated plants indicated an outcrossing system, with some self-fertility, and an S_1 progeny with inbreeding depression (Connor and Cook, 1955). Further studies show that inbreeding depression occurs in other collections, but that when S_1 plants are allowed to outcross with open-pollinated lines there is a full recovery of vigour. Results are summarised in Table 1 for S_1 and S_2 and open-pollinated generations of the "Tara Hills" collection.

In the S_1 and S_2 of the "Tara Hills" collection there was no obvious sorting into genotypes, as was the experience with the S_1 from the Molesworth collection.

TABLE 1. MEAN PLANT HEIGHT AND NUMBER OF CULMS IN SELFED AND OPEN-POLLINATED *F. novae-zelandiae* FROM "TARA HILLS," OMARAMA

Season	1956–57			1958–59					
Generation	S_1	O–P	No. of Plants	S_2	O–P	No. of Plants	(S_1) O–P	O–P	No. of Plants
Mean Height Culms (cm)	108.7	119.7*	41	70.1	83.9*	88	89.2	84.5	88
Mean No. of Culms	196.1	302.5*	41	44.5	166.2*	88	150.0	167.6	88

*Significant at 0.1% level

One S_1 plant of *F. novae-zelandiae* from the Molesworth collection was permitted to outcross with the open-pollinated line. Progeny from this plant was compared with plants from continual open-pollination. Here there was an increase in vigour after outcrossing following selfing, over the continually open-pollinated group. The mean height for open-pollinated plants was 98.0 cm, and 100.3 cm for plants from outcrossing after selfing, while mean number of culms was 183.6 for the former 231.6 for the latter.

The conclusion can again be drawn that *Festuca novae-zelandiae* is an outcrossing species.

Festuca matthewsii (HACK.) CHEESEMAN. In November 1957 four plants of this indigenous perennial species were selfed. These plants were from the Awakino River, North Otago, and from Handyside Stream, North Canterbury. All four plants set seed on selfing and the percentages are indicated in Table 2. Germination was poor (cf. Table 2) and only 54 plants were finally available for planting in a trial; these were from the selfing of G1161 from Handyside Stream, North Canterbury.

TABLE 2. SEED SET AND GERMINATED IN *F. matthewsii* SELFED 29.11.57

Collection	No. seed set	Total florets	% Seed set	No plants obtained	% Germination	Locality
G1147	26	505	5.1	5	19.2	Awakino River
G1147	136	519	26.2	5	3.7	Awakino River
G1159	20	644	3.1	0	0	Handyside Stream
G1161	161	684	25.5	58	36.0	Handyside Stream
TOTAL	343	2352	14.6	68	19.8	

Like *F. novae-zelandiae*, *F. matthewsii* does not flower until its second year. Inbreeding depression was noticeable both in the height of the plants and in the number of culms produced: again there was no evident segregation. The results from comparison of selfed and open-pollinated progenies are given in Table 3. It is concluded that *F. matthewsii* is an outcrossing species. This is in keeping with the general observation that perennial species of *Festuca* normally outcross (Nilsson, 1934).

TABLE 3. Mean Height and Mean Culm Number in *F. matthewsii* S_1 and Open-pollinated Generations

Generation	Mean height (cm.)	Standard deviation	Mean No. culms	Standard deviation	No. of plants
Open-pollinated	64.9*	8.01	70.8*	66.5	52
Self-pollinated	53.4	5.43	15.7	17.8	47

*Significant at 0.1% level

Festuca multinodis Petrie et Hack. A perennial species found only in New Zealand. Three plants of this species, from a collection made from Point Gordon, Wellington Harbour, have been selfed, one in 1954–55 season and two in 1956–57 season, but in none of the selfings has seed been set. It is concluded that *F. multinodis* is self-sterile.

Poa colensoi Sprengel. It was previously reported (Connor, 1957) that *P. colensoi* is self-sterile after selfing had been made on eight plants from various localities. One further selfing of a plant from the Ashburton Gorge supports this original conclusion, while Ashburton Gorge plants were readily control-crossed with plants from Central and North Otago.

Tribe Aveneae

Koeleria kurtzii Hack. This perennial species is thought to occur in Argentina and possibly in Australia but the taxonomy of the group is confused. Two collections were available: *K. kurtzii I*, raised from seed from North Canterbury; and *K. kurtzii II*, raised from seed collected at Omarama, North Otago. Morphological differences between the two collections were quite marked. A plant of *Koeleria kurtzii I* was selfed in 1952–53 season and yielded no S_1 plants, neither did a plant selfed in 1953–54. A single plant was selfed in 1957–58 season which yielded sufficient S_1 progeny for a trial. In *Koeleria kurtzii II* two plants were selfed in 1954–55 but there was no S_1 progeny. A single plant selfed in 1955–56 season yielded a small S_1 which was planted out in a trial. A further selfing in 1957–58 yielded an S_1 population large enough for a further trial. The results from measurements on the three trials in *K. kurtzii* are presented in Table 4.

There is no evidence for inbreeding depression in the three trials examined, and it must be concluded that the species is self-fertile, even

TABLE 4. MEAN HEIGHT AND MEAN CULM NUMBER IN *Koeleria kurtzii* S_1 AND OPEN-POLLINATED GENERATIONS

Collection	*K. kurtzii I*		*K. kurtzii II*			
Season	1959–60		1957–58		1959–60	
Generation	S_1	O–P	S_1	O–P	S_1	O–P
Mean plant height (cm.)	61.6	60.3	52.8	58.3*	55.7	54.7
Mean No. culms	328.4	308.0	182.7	176.2	420.0	399.8
No. of plants	25	25	13	13	30	30

*Significant at 5% level.

though four plants gave no S_1 progeny in the seven plants selfed. Although these two collections grew in adjacent rows and there were marked morphological differences between them good enough for conspicuous genetic markers there was no evidence in the open-pollinated generations from either collection that there had been any outcrossings. Smith (1944) reported that *K. cristata* (L.) Pers. set 0.1% seed under self-pollination.

Tribe Agrostideae

Deyeuxia billardieri KUNTH. A perennial species said to be common in Australia and Tasmania (Cheeseman, 1925, p. 161). Two plants of this species in a collection raised from seed from Waiwera in Rodney County, were selfed in January 1957. S_1 progenies were obtained from both plants which were planted with open-pollinated plants as controls in a randomised plot. In this species the number of flowering culms per plant was counted, and the plants also scored in five grades of vigour. Details are presented in Table 5.

TABLE 5. VIGOUR AND MEAN NUMBER OF CULMS IN *Deyeuxia billardieri* S_1 AND OPEN-POLLINATED GENERATIONS

Vigour Score	No. of Plants	
	S_1	O–P
5	1	0
4	14	2
3	61	77
2	8	4
1	0	1
Total	84	84
Mean Vigour Score	3.1	3.0
Mean No. Culms	24.8	22.5

In this trial the vigour values were based on leafiness and tillering. Tillering affects the production of flowering shoots and as a weak plant with few tillers will have only few flower heads, the agreement for mean vigour score and mean number of culms can therefore be expected and is perhaps only one effective measurement.

In this trial selfing for one generation had no effect on the progeny and the species can therefore be considered self-fertilising.

Tribe Danthonieae

Danthonia. Collections of species of the tall tussock Danthonias were available for selfing but only a little has been done. Difficulties in germinating seed were encountered early in the study of these species and these have only recently been overcome. Data are presented in Table 6 of seed-set under selfing in two species tested and some seed-set percentages from open-pollination are also presented. Trials on this species can be expected to take several years as plants raised from seed do not effectively come into flower until they are four years old.

Nearly 10,000 florets were examined in *D. rigida** for seed-set under open-pollination and 2,600 florets from the selfings, so that some of the high seed-set values recorded from open-pollination can be taken to represent the potential seed-setting capacity of this species.

Preliminary results show that the two plants of *Danthonia cunninghamii* tested were self-sterile, while two plants of *D. rigida* showed some self-fertility.

TABLE 6. SEED SET IN *Danthonia* SPP.

Species	Treatment	Seed Set (%)
	Garden Grown	
Danthonia cunninghamii Hook. f. G.459, G.623	Selfing 2 plants	Nil
	Open-pollination	Liberal Seed Set
D. rigida Raoul G.556	Selfing	48.8
G.567	Selfing	16.5
G.567	Open-pollination	80.6
G.29	" "	18.6
G.555	" "	39.2
G.568	" "	39.2
G.569	" "	55.5
G.30	" "	85.4
	Field Collections from South Canterbury	
Hunters Hills	Open-pollination	34.9
Coal Hill	" "	27.9
"Pukaki Downs"	" "	20.9
Burkes Pass	" "	13.5

**Danthonia rigida* Raoul *sensu stricto*, is a narrow-leaved snow-tussock; red tussock is not included under this name

Discussion

In the four species examined in detail two were self-fertile and the S_1 progenies showed no inbreeding depression while *Festuca matthewsii* and *F. novae-zelandiae* were somewhat self-fertile, but with low germination of S_1 seed and with inbreeding depression in the S_1 progeny. In these two species there are no mechanisms to prevent selfing and there are still no indications of the frequency with which selfing occurs in nature.

Deyeuxia billardieri is listed by Cockayne and Allan (1934) as possibly crossing with *D. forsteri* but although these species were growing close together in the trials no apparent hybrids are present in the later progenies of these species.

The extent to which self-fertile species are present in the 21 species examined is of some interest though these may not be a representative enough sample of the indigenous grasses. Including the results from this paper there are 13 species which are self-fertile with normal selfed progenies. Three species yield degenerate progenies on selfing and four species are self-sterile, while there are no data on the self-fertility of the gynodioecious *Arundo richardii*.

Conclusions

The results from these investigations are summarised:

(1) Self-fertile with normal selfed progeny: *Deyeuxia billardieri*, *Koeleria kurtzii*.

(2) Self-fertile but with degenerate selfed progeny: *Festuca novae-zelandiae*, *F. matthewsii*.

(3) Self-sterile. *Festuca multinodis*.

Acknowledgments

My thanks are due to Miss L. B. Moore, Messrs G. A. Dunbar, J. Stone, and A. P. Druce for collections of plants and seed used in some of these trials, and to Mr A. Rowell for collections of *Danthonia rigida* panicles from South Canterbury.

References

Cheeseman, T. F. 1925: "Manual of the New Zealand Flora". 2nd Ed. Govt. Printer. Wellington.

Cockayne, L.; Allan, H. H. 1934: An Annotated List of Groups of Wild Hybrids in the New Zealand Flora. *Ann. Bot. 47:* 1–55.

Connor, H. E. 1954: Studies in New Zealand *Agropyron*. *N.Z. J. Sci. Tech. B35:* 315–43.

———— 1957: Breeding Systems in Some New Zealand Grasses. *Ibid. A38:* 742–51.

Connor, H. E.; Cook, Audrey B. 1952: Note on the Breeding System of *Dichelachne crinita* (Linn.f.) Hook.f. in New Zealand. *Ibid. A34:* 369–71.

———— 1955: The Breeding system of New Zealand Fescue-tussock *Festuca novae-zelandiae* (Hack.) Cockayne. *Ibid. A37:* 103–5.

Connor, H. E.; Penny, E. D. 1960: Breeding Systems in New Zealand Grasses II. Gynodioecy in *Arundo richardii* Endl. *N.Z. J. Agric. Res.*

Nilsson, F. 1934: Studies in Fertility and Inbreeding some Herbage Grasses. *Hereditas, 19:* 1–162.

Smith, D. C. 1944: Pollination and Seed Formation in Grasses. *J. Agric. Res. 68:* 79–95.

EFFECTS OF GIBBERELLIC ACID, INDOLE-ACETIC ACID, COUMARIN AND PERLOLINE ON PERENNIAL RYEGRASS (*Lolium perenne* L.)

By S. O. Fejer, Grasslands Division, Department of Scientific and Industrial Research, Palmerston North

(*Received for publication, 11 May 1960*)

Summary

The effects of several growth substances on growth and tillering of perennial ryegrass were investigated.

Gibberellic acid produced initial elongation resulting in taller plants, followed by total reduction due to reduced rate of tillering, as reported by other workers. At the rates used, there was no promotion of flowering, either in the sense of replacement of vernalization or day-length requirement, or in earliness. Inbreeding depression of green weight was not removed by gibberellic acid, but there were some responses, differing with the genotype.

Coumarin was found to have little effect on growth or tillering.

The main effect of indole-acetic acid was the rooting promotion of derooted cuttings.

Perloline had a growth-inhibiting effect, not reported previously.

Interactions were found between the treatments and genotypes.

Introduction

There is a growing body of literature on the effects of gibberellic acid (GA), indole-acetic acid (IAA) and coumarin on the growth and development of plants but relatively little is known on intra-specific variations in growth responses to such materials. Differential responses to applied auxins have been reported by Nagel (1939) in *Nicotiana* and the implications to biometrical genetics and to measurements of subspecific divergence have been discussed by Anderson and Ownbey (1939) and by Gregor (1956). Phinney (1956) reported that GA might offset the effects of some responsive dwarfing genes in maize, and similar observations were made in barley by Stoy and Hagberg (1958), and in perennial ryegrass by Cooper (1958). Coumarins have been reported by Pollock *et al* (1954) to inhibit the growth of roots, while perloline, a fluorescent alkaloid discovered by Grimmet and Melville (1942) in perennial ryegrass, was observed to be in relatively high amounts in some dwarf or non-vigorous plants (Dr. J. Mangan, formerly Plant Chemistry Division, Palmerston North, pers. comm.).

In the present trials, these four substances were applied to plants of perennial ryegrass, representing extremes in type of productivity and tiller number. Differences in response to vernalisation have already been established between different tillering types (Fejer, in preparation), and as vernalisation requirements (Napp-Zinn, 1956) and tillering (Leopold, 1949) have both been reported to be related to growth substance metabolism, it was considered that any differential response obtained in the present trials would assist in the separation of this plant material on productivity and tillering characteristics.

Experimental Methods and Results

Most of the plants used in this study came from different stages of a selection and inbreeding programme for high and low herbage production in perennial ryegrass (Fejer, 1955) and are referred to as "H" and "L" respectively. The selection cycle is shown by a number, e.g., L3 represents the third generation of selection for low herbage production in sib-crosses, and if produced by selfing by the letter "S", e.g., HS4 represents the fourth selfed generation from a high-producing selection. Some plants used differed in perloline content and others contained a floral abnormality (gene "mo" = multi-ovary) from a "H" inbred line (Fejer 1958).

Four separate experiments were carried out: (1) To determine the effects of GA on growth and flowering of unvernalised seedlings grown under contrasting daylengths; (2) To determine the effect of GA on growth and flowering of vernalised clonal material grown under natural early spring conditions; (3) To study the effects of IAA, perloline and coumarin on productivity and tillering habits when injected into tillers of selected clones; (4) To study the differential effects of these three substances at various concentrations when taken up by the roots or by the tiller bases after the removal of the roots.

Experiment 1. The effects of GA on unvernalised seedlings under different daylengths

Seeds were sown on 25 April 1957 in 4-in. plastic pots and the plants kept in a heated glasshouse under natural daylength. On 9 July, plants were transferred to two glasshouses kept at the approximate mean temperature of 50°F by night and 70°F by day, one with normal daylength increasing from 10 to 13 hours during the experiment (ND), and the other with an artifically extended day of 16 hours (LD). In each glasshouse, two plants of LS4, three of HS4, one of "mo" and one of HS3 × LS3 progenies were treated with GA and the same number left untreated. One droplet of water (approx. 0.05 cc) containing GA at a 10^{-4} g/cc concentration was placed in the axil of the lowest leaf of each tiller. The GA treatment continued weekly until the first ears started to emerge from the leaf sheath on 28 August. By then the average amount of GA received by the plants was 0.04 mg. The actual amount varied with the tiller number of the plants. The pots received Hoagland's nutrient solution several times to ensure good response to GA (Leben and Barton, 1957).

Results: The normal outcross HS3 × LS3 served mainly as a check on the growth of the inbred lines and has been excluded from the statistical analyses because of its higher production. Table 1 shows that the growth of all inbred material was strongly depressed compared with that of the outcross. One effect of GA could be to counteract these inbreeding effects if caused by GA-sensitive recessive genes uncovered by four generations of inbreeding. Treatment of the inbred lines with GA increased plant height but decreased tillering rate so that green weight showed no response. Even in plant height the GA treated inbreds did not attain the height of the untreated outcrossed plants.

TABLE 1. Response of Perennial Ryegrass to Gibberellic Acid

Progeny Treatment	Plant per Group	Plant Ht. (cm) 31 July	Plant Ht. (cm) 21 Aug.	Green Wgt. (g) 27 Sept.	Tiller Number 27 Sept.
LS4					
(LD)	2	22	38	4.8 FF	27
(LD + GA)	2	29	50	3.8	9
(ND)	2	19	30	4.9 F	16
(ND + GA)	2	30	49	3.1 F	12
SE ±		2.8	3.9	1.3	4
HS4					
(LD)	3	21	34	8.9	33
(LD + GA)	3	25	34	7.9	33
(ND)	3	19	29	7.4	33
(ND + GA)	3	28	46	7.8	26
SE ±		2.3	3.2	1.0	3
"mo"					
(LD)	1	17	29	3.7	28
(LD + GA)	1	32	56	6.5	18
(ND)	1	16	28	2.7	14
(ND + GA)	1	23	37	3.0	14
SE ±		4.0	5.5	1.8	6
HS3 × LS3					
(LD)	1	26	42	8.8 F	29
(LD + GA)	1	30	60	9.8 F	13
(ND)	1	26	48	14.0 F	30
(ND + GA)	1	35	66	11.0 F	23

"F" represents one flowering plant

In both July and August, the LS4 plants showed greater increase in height than did the HS4 plants which showed little response to GA in this respect, particularly under long-day treatment. Generally, plants under ND were shorter than those under LD, although the difference was statistically significant only in August. In some cases the reduction attributable to the normal-day treatment has been lessened or reversed by GA. The opposite effect occurred in the "mo" progeny.

Since it is not known whether floral primordia were present when GA was first applied and since flowering was irregular in the LS4 and HS3 × LS3 progenies, no conclusions can be made on the effect of GA on ear initiation and development, and no such effects were observed. The result reported previously by Knapp (1956) working with *Gallinsoga* and *Agrostemma*, that GA extends the period between inflorescence emergence and flowering under short days, was also found in this study under normal days. The length of this period was doubled from 8 to 16 days in plants under ND, but shortened from 22 to 12 days in the one case where GA-treated plants flowered under LD (in HS3 × LS3).

Experiments 2. The effects of GA on elongation and flowering of vernalised clones under natural early spring conditions

The main purpose of this experiment was to observe the effect of GA on ear emergence and on date of flowering as measured by the onset of anthesis. Cuttings from two plants of the HS4 and LS4 progenies

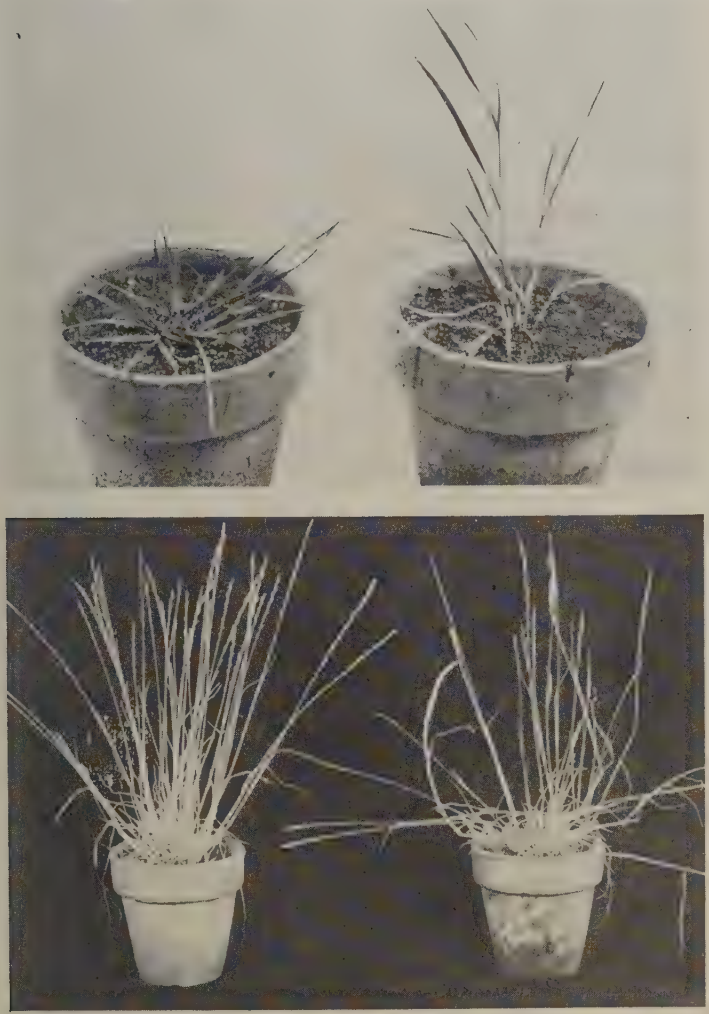

Fig. 1.—GA treated (right) and untreated (left) cuttings of LS4 perennial ryegrass
(a) before ear emergence
(b) at maturity.

used in the previous experiment were grown in soil, in 6-in. clay pots. One cutting of each was treated with GA by the method used in the first experiment, between 12 September and 23 October 1957 (total amount of GA applied, approximately 0.03 mg) and the other untreated. The plants were grown outdoors and exposed in the winter to normal cold temperatures and to normal increasing daylengths.

Results: All LS4 plants, whether treated or not, flowered between 20 and 24 November while the HS4 plants flowered on 8 December, with slightly more variation in the preceding ear emergence dates. This shows clearly that GA had no effect on flowering date.

Because of the low numbers of plants and cuttings used, no data are presented, and only photographs of one LS4 clone are shown in Figs. 1 (a) and (b).

They show the spectacular response to GA in tiller elongation before the onset of ear emergence, and in the reduction of number of heads at flowering. Although, in the LS4 plants, weight was reduced by GA through reduction of tillering, HS4 plants did not show this effect. Another difference in the response to GA was in numbers of nodes which were increased in the LS4 plants while unchanged or decreased in the HS4 plants. Other effects observed after GA application were a tendency to branching both in the lower nodes and in the flowering heads, and an extreme elongation of the lower internodes, causing the lodging effect seen in Figs 1 (a) and (b).

Experiment 3. Effects of injection of IAA, perloline and coumarin

The material used in this experiment was derived from cuttings of two plants of L3, one of H3 origin, and three plants having different perloline contents (Mangan, unpublished). These cuttings were grown in soil in 6-in. clay pots, one cutting of each plant being placed in a heated glasshouse and another in an unheated one on 22 June 1956.

Two test-substances, IAA and perloline, and water were then injected into the tiller bases of three separate cuttings of each plant, by the method described by Leopold (1949). IAA was used at a concentration of 2×10^{-2} g/cm^3 and perloline at 2×10^{-2} g/cm^3. In the second L3 plant perloline was replaced by coumarin at a concentration of 2×10^{-4} g/cm^3.

The experiment was harvested on 6 August 1956.

Results: Table 2A shows the results for the plants chosen for varying perloline content.

As Mangan (pers. comm.) found, there was a difference between these plants in green weight over the three treatments, the "high perloline" plant weighing 6.5 g and the other two 11.1 and 12.1 g ($F = 6.86$ for 2.15 d.f.; $p = 0.01$). The ratio of tiller number to tiller weight (tiller index), shown in the table, was increased ($p = 0.07$) by the perloline and IAA applications, but interactions between application and original perloline content did not reach significance level.

In Table 2B the green weights of the plants selected for herbage production show that there was a significant interaction between genotype

TABLE 2. TILLER INDICES AND GREEN WEIGHTS OF PERENNIAL RYEGRASS PLANTS INJECTED WITH TEST SUBSTANCES AND WATER

A. Tiller Indices (Tiller Number/Tiller Weight in g.) of 3 plants with different perloline contents

Plant	Treatment	Warm House	Cold House
High Perloline	(IAA)	433 (65 / 0.15)	600 (54 / 0.09)
	Perloline	500 (60 / 0.12)	520 (52 / 0.10)
	Water	327 (49 / 0.15)	325 (39 / 0.12)
Intermediate	(IAA)	163 (49 / 0.30)	393 (55 / 0.14)
	Perloline	395 (79 / 0.20)	350 (42 / 0.12)
	Water	175 (63 / 0.36)	283 (46 / 0.16)
Low Perloline	(IAA)	379 (72 / 0.19)	578 (52 / 0.09)
	Perloline	495 (94 / 0.19)	913 (73 / 0.08)
	Water	395 (79 / 0.20)	727 (80 / 0.11)

B. Green weights of 3 plants selected for herbage production (g)

Plant	Treatment	Warm House	Cold House
H3	(IAA)	16.0	8.5
	Perloline	16.0	7.5
	Water	22.5	17.0
L3 (1)	(IAA)	10.5	3.5
	Perloline	12.0	5.0
	Water	6.5	3.0
L3 (2)	(IAA)	11.0	8.5
	Coumarin	15.0	12.0
	Water	8.0	6.0

The plant x treatment interaction was significant at the 5% level ($F = 5.91$ for 1;8 d.f.)

and treatment. The H3 plant was depressed by both IAA and perloline while the L3 plants were stimulated by all three materials.

Experiment 4. The effects of root-soaking with IAA, perloline and coumarin

Seventy individual tillers of each of one "H" and one "L" non-inbred plant were soaked at the base for 24 hours, in August 1956, five with roots attached and five with roots removed, in solution of each of the following substances:

	Concentration, g/cc	
	High Rate	Low Rate
IAA	10^{-5}	10^{-6}
Perloline	10^{-4}	10^{-5}
Coumarin	10^{-4}	10^{-5}
Water Control		

TABLE 3. DRY WEIGHTS OF HERBAGE AND ROOTS, TILLER NUMBERS AND WEIGHTS OF PERENNIAL RYEGRASS PLANTS, BASE-SOAKED IN TEST SUBSTANCES OF DIFFERENT CONCENTRATIONS, WITH ROOTS ATTACHED OR REMOVED

A. Chemical treatment x Genotype

(1) Dry Weight per Tiller (mg) (p = 0.01)

	Water	(IAA)	Perloline	Coumarin	
"H" selection	49.2	51.2	44.7	49.0	Sig. diff.
"L" selection	56.8	48.2	64.2	55.0	5% = 6.6

B. Chemical treatment x Root treatment

(1) Dry Weight of Herbage per Plant (mg) (p = 0.01)

	Water	(IAA)	Perloline	Coumarin	
Roots attached	706	533	747	610	Sig. diff.
Roots removed	572	574	462	519	5% = 121

(2) Dry Weight of Roots per Plant (mg) (p = 0.05)

	Water	(IAA)	Perloline	Coumarin	
Roots attached	320	259	345	307	Sig. diff.
Roots removed	286	240	219	247	5% = 59

(3) Tiller Number per Plant (p = 0.05)

	Water	(IAA)	Perloline	Coumarin	
Roots attached	12.3	11.5	13.4	11.3	Sig. diff.
Roots removed	12.3	11.8	9.4	10.5	5% = 2.4

C. Chemical treatment x Rate

(1) Dry Weight of Herbage per Plant (mg) (p = 0.10)

	(IAA)	Perloline	Coumarin	
High Rate	480	627	575	Sig. diff. 5% = 121
Low Rate	626	582	553	

D. Root treatment x Rate

(1) Dry Weight of Roots per Plant (mg) (p = 0.05)

	Roots attached	Roots removed	
High Rate	320	217	Sig. diff. 5% = 49
Low Rate	287	254	

The five replications were laid down in randomized blocks. Tops and roots were cut to a uniform length and the cuttings were planted in 4-in. plastic pots filled with vermiculite and watered regularly with Hoagland's

nutrient solution. They were grown in the spring of 1956, in an unheated glasshouse, and harvested in November.

Results: As in Exp. 3, it was not possible to discriminate between the individual substances in total effect, but the depression of herbage weight by their combined effect approached significance level ($p = 0.15$).

A number of significant interactions between individual chemicals or their rates of application with the plant material have been calculated and are shown in Table 3.

An outstanding interaction—that between the chemical treatment and genotype—was highly significant in tiller weight which was lowered by IAA and increased by perloline in the "L" plant only (A). A second interaction—that between chemical treatment and root treatment—for the characters of herbage weight, root weight and tiller number (B) can be explained by the root-promoting property of IAA manifested in the plants without roots, while perloline was particularly inhibiting to these same plants. No recordings were made of type and number of roots. On the other hand, the narrow optimum concentration for auxin applications is reflected in the chemical treatment × rate interaction (C), as a rate difference in herbage weight appeared only in the IAA treatment. The combination of this narrow optimum with the root-promoting property is shown in the root-treatments × rate interaction in root weights (D).

Discussion

The results obtained from applications of GA on perennial ryegrass agree with those reported by Morgan and Mees (1956) who found temporary high and ultimate low production in GA treated grasslands. There was a spectacular temporary increase in height through elongation of the leaves and of the basal internodes but at the same time a decrease in tillering resulted finally in production lower than that from untreated plants. The temporary increase in height after GA was applied was relatively higher in Experiment 2 than in Experiment 1. Under the colder conditions of Experiment 2 GA acted as if breaking the dormancy in growth, as reported by Morgan and Mees, but the use of GA for out of season production is not practical as Scott (1959) recently confirmed under New Zealand winter conditions, that the depression in growth following the initial stimulation was quite serious when GA was applied to pastures.

It is noteworthy that in perennial ryegrass which requires vernalisation and long days for flowering, GA at the concentrations used did not promote flowering under any of the experimental conditions.

Of the other substances compared, the general growth inhibiting activity of perloline, whether applied or natural, is in conformity with an earlier finding on the inhibition of root growth by this substance in the cress root growth test (Fejer, in preparation).

Of the many known properties of IAA, only the promotion of rooting of cuttings was observed in these experiments. As Leopold (1955) has pointed out, the activity of such substances is so delicately

balanced that even the slightest changes in environment or other factors can reverse their effects from one extreme to the other. This could also explain why the contrasting effects of auxin and coumarin on tillering and apical dominance, reported by Leopold (1949), were not substantiated in the present experiments.

Some of the results give support to the supposition that there is a difference between ryegrass plants in their response to different chemicals. Both Exp. 1 and 2 showed that the "L" plants were more susceptible than the "H" plants to the initial elongation as well as to the final depression in yield caused by GA applications, although if there were any differences between the plants in the amount of GA recevied per unit plants tissue due to the application of the same amount to each tiller, the "L" plants with the larger tillers would have received the relatively lower amounts. This could well be because of genetic differences in the level of gibberellin-like substances, the general occurrence of which has been shown by Phinney and West (1956). In Exp. 4, applications of IAA reversed the relationship in tiller weight usually found between "H" and "L" selections, possibly by interfering with the natural auxin metabolism.

Acknowledgments

The writer wishes to acknowledge help in biometrical problems by Mr A. C. Glenday, Applied Mathematics Laboratory, and in measurements by Mr P. H. Drake, Grasslands Division, Department of Scientific and Industrial Research; the photographs were made by the late Mr H. Drake.

References

Anderson, E.; Ownbey, Ruth P. 1939: The Genetic Coefficients of Specific Difference. *Ann. Mo. bot. Gdn. 26:* 325.

Cooper, J. P. 1958: The Effect of Gibberellic Acid on a Genetic Dwarf in *Lolium perenne*. *New Phytol. 57:* 235.

Fejer, S. O. 1955: Genotype-environment Interaction in *Lolium perenne*. *Nature, Lond. 175:* 944.

———1958: A Gene for Femaleness in Hermaphrodite *Lolium perenne* L. *Proc. 10th int. Congr. Genet.* p.81.

Gregor, J. W. 1956: Genotypic-environmental Interaction and its Bearing on a Practical Problem of International Interest. *Proc. 7th int. grassl. Congr.* p. 202.

Grimmett, R. E. R.; Melville, J. 1942: A Fluorescent Alkaloid in Ryegrass (*Lolium perenne* L.) *N.Z. J. Sci. Tech. B24:* 149.

Knapp, R. 1956: Ueber die Wirkung von Gibberellin auf Wachstum und Bluten- bildung bei verschiedenen Temperatur—und Lichtverhaltnissen. *Z. Naturforsch. 11b:* 698.

Leben, C.; Barton, Lela V. 1957: Effect of Gibberellic Acid on Growth of Kentucky Blue Grass. *Science, 125:* 494.

Leopold, A. C. 1949: The Control of Tillering in Grasses. *Amer. J. Bot. 36:* 437.

———1955: "Auxins and Plant Growth". Univ. Calif. Press. Berkeley. 354 pp.

Morgan, D. S.; Mees, G. C. 1956: Gibberellic Acid and the Growth of Crop Plants. *Nature, Lond. 178:* 1356.

Nagel, Lilian 1939: Morphogenetic Differences between *Nicotiana alata* and *N. langsdorffii* as indicated by their Response to Indoleacetic Acid. *Ann. Mo. bot. Gdn. 26:* 349.

Napp-Zinn, K. 1956: Zur Frage nach der Uebertragbarkeit des durch die Vernalisation bewirkten Bluhimpulses. *Ber. dtsch. bot. Ges. 69:* 193.

Phinney, B. O. 1956: Growth Response of Single Gene Dwarf Mutants in Maize to Gibberellic Acid. *Proc. nat. Acad. Sci. Wash. 42:* 185.

Phinney, B. O.; West, C. A. 1956: The Growth Response of Single Gene Dwarf Mutants of *Zea mays* to Gibberellins and to Gibberellin-like Substances. *Proc. int. Genet. Symp., Cytologia Suppl.* p.384.

Pollock, B. M.; Goodwin, R. H.; Greene, Susan 1954: Studies on Roots. II. Effects of Coumarin, Scopoletin and other Substances on Growth. *Amer. J. Bot. 41:* 521.

Scott, R. S. 1959: Effect of Gibberellic Acid and Nitrogen on Winter Growth of Pasture... *N.Z. J. agric. Res. 2:* 1203.

Stoy, V.; Hagberg, A. 1958: Effects of Gibberellic Acid on Erectoides Mutations in Barley. *Hereditas, 44:* 516.

NEW ZEALAND JOURNAL OF SCIENCE

Volume 3, No. 2, June 1960.

CONTENTS

Primary Production in a New Zealand West Coast Phytoplankton Bloom. *R. M. Cassie* and *V. Cassie.*

Establishment of *Proceidochares utilis* Stone (Diptera: Trypetidae) on *Eupatorium adenophorum* Spreng. in New Zealand. *J. M. Hoy.*

Sulphur Isotopic Variations in Nature. Part 7. Sulphur Isotopic Measurements on Sulphur and Sulphates in New Zealand Geothermal and Volcanic Areas. *T. A. Rafter, I. A. Kaplan* and *J. R. Hulston.*

Physiological Studies of some Common Fungi from Grassland Soils. *D. J. Ross.*

Parasites of the European Rabbit, *Oryctolagus cuniculus* (L), on some Subantarctic Islands. *P. C. Bull.*

Pelagic Amphipoda of the N.Z.O.I. Pacific Cruises, March 1958. *D. E. Hurley.*

Breadmaking without Bulk Fermentation: The Effect of Mixing and Stage of Addition of Potassium Iodate. *T. A. Mitchell* and *C. A. Crawford.*

A Leaf Squash Technique for Chromosome Studies in Grasses. *G. de Lautour.*

Limitations Imposed by some Waterborne Preservatives on the Soil Block Test Method. *A. J. McQuire.*

The Alpine Grasslands of the Hokitika River Catchment, Westland. *M. J. Wraight.*

A Modified Teichert Method for the Estimation of Fat in Meat. *W. V. Falkenhahn.*

Sulphur Isotopic Variations in Nature. Part 8. Application to some Biogeochemical Problems. *I. A. Kaplan, T. A. Rafter* and *J. R. Hulston.*

SUBSCRIPTIONS. Subscriptions for the New Zealand Journal of Agricultural Research will be accepted only on advance payment and only for a full calendar year. Price 45s. per annum; single copy 7s. 6d., post free (surface mail). Published in alternate months, starting February. Subscriptions should be forwarded to the Publications Officer, Department of Scientific and Industrial Research, P.O. Box 8018, Wellington, New Zealand. Cheques should be made payable to the Department of Scientific and Industrial Research, and if drawn on banks outside Wellington, must have exchange added. Subscribers are requested to give their name and full postal address.

This Journal may also be ordered through The High Commissioner for New Zealand, 415 Strand, London W.C.2, or through any bookseller.

ERRATUM. The prices given in the February issue were incorrect, and should be as above.

CONTENTS

PAGE

Preliminary Assessment of Toxaphene, Stronbane and Thiodan for Control of Clover Case-Bearers (*Coleophora* spp.) (Coleophoridae, Lepidoptera). *J. M. Hoy* 617

Yeasts from Soils under Forest and under Pasture. *M. E. di Menna* 623

Development of a Method for Routine Testing of Poultry Feeds. *Jean G. Miller* and *J. Edmondson* 633

Winter Growth of Lucerne (*Medicago sativa*). II. Genetic Variations in a Population of Hunter River Lucerne. *T. P. Palmer* 641

Growth of Pasture Species in Controlled Environment. II. Growth at Low Temperatures. *K. J. Mitchell* and *Renate Lucanus* 647

Response of Some New Zealand Pasture Species to Vernalization. *S. O. Fejer* 656

Changes in the Pore-Space of a Pasture Topsoil under Animal Treading. *M. W. Gradwell* 663

Control of Superficial Scald on Granny Smith Apples Using Pre-Harvest Sprays of Diphenylamine. *C. A. S. Padfield* and *R. M. Smock* 675

Evaluation of General and Specific Combining Ability in a Late-flowering Variety of Red Clover (*Trifolium pratense* L.). *L. B. Anderson* 680

Scale Insect Survey on Apples 1959-60. *Aola M. Richards* 693

Fungi of Some Forest and Pasture Soils. *R. H. Thornton* 699

Photosensitivity Diseases in New Zealand. XXII. Studies in the Rabbit on the Effect of Injection of Extracts Containing Facial Eczema Toxin. *N. A. Worker* and *D. C. Dodd* 712

Breeding Systems in New Zealand Grasses.

II. Gynodioecy in *Arundo richardii* Endl. *H. E. Connor* and *E. D. Penny* 725

III. Festuceae, Aveneae and Agrostideae. *H. E. Connor* 728

Effects of Gibberellic Acid, Indole-acetic Acid, Coumarin and Perloline on Perennial Ryegrass (*Lolium perenne* L.). *S. O. Fejer* 734

Printed by Coulls Somerville Wilkie Ltd.,
Wellington, New Zealand.